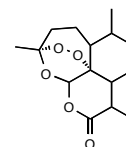
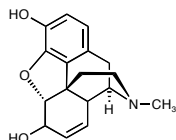




Phytochemical Society of North America

Société Phytochimique de L'Amérique du Nord • Sociedad Fitoquímica de América del Norte



51st Annual Meeting of the **Phytochemical Society of North America**

August 11-15, 2012

The University of Western Ontario
London, ON, Canada

Program & Abstracts



Welcome to London Ontario, Western University and PSNA 2012!

We are excited about the great line up of invited speakers we have assembled for the 51st Annual Meeting of the Phytochemical Society of North America. Five symposia that largely define the field of Phytochemistry have been organized, with each one led by presentations from internationally recognized leaders. These include symposia on *Biosynthesis & Metabolism*, with featured talks on vitamin C by Argelia Lorence, and enzyme specificity by Kevin Walker, *Genomics & Bioinformatics*, with featured talks on quantitative genomics by Daniel Klipenstein and metabolic diversity by Anne Osbourn, *Botanicals & Medicinals*, with featured talks on phytochemical complexity by Paula Brown and metabolic syndrome by Ilya Raskin, and *Phytochemicals in the interaction between plants and their environment*, with featured talks on below ground terpene metabolism by Dorothea Tholl and steroidal glycoalkaloids by Jim Tokuhisa. A fifth symposium, *Bioproducts From Canadian Forests: Production of Valued Attributes*, will feature talks on bioproduct research & development in Canada by Tom Rosser, medicinal plants by John Arnason, bio-oil and bio-char by Franco Berruti, enzyme conversion of forest products into high value polymers by Emma Master and conifer triterpenes by Philipp Zerbe. Each symposium is rounded out by a wide range of presentations chosen from submitted abstracts. Finally, we have an excellent collection of posters assembled comprehensive poster session during the week.

We hope you enjoy PSNA 2012!

Organizing Committee

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The University of Western Ontario

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Sponsorship

The organizers of the PSNA 2012 Annual General Meeting gratefully acknowledge the financial support provided by the following institutions:



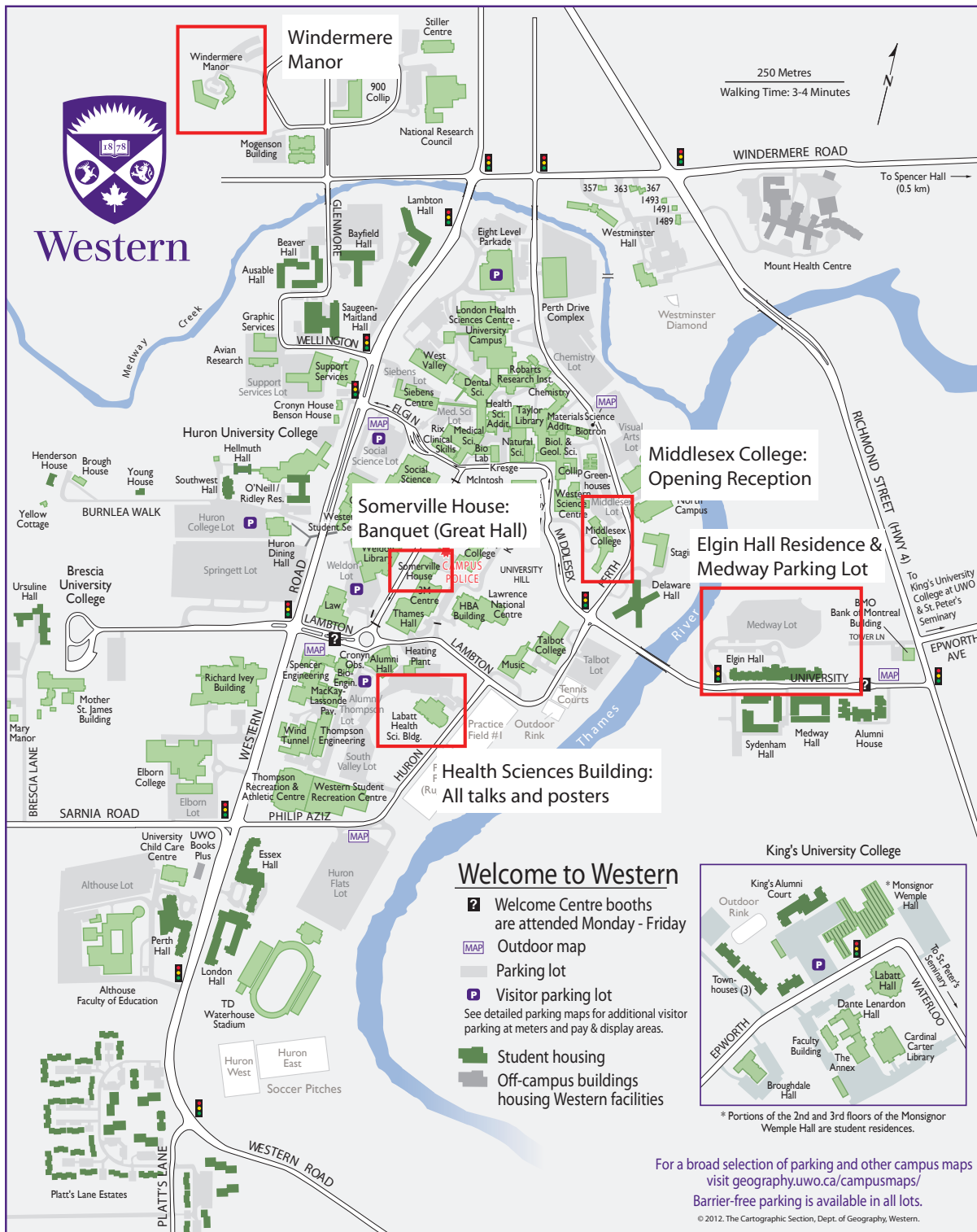
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In-kind support for advertisement and abstract publication was generously provided by Elsevier and *Pharmaceutical Biology*, respectively.

Western University Campus Map
Buildings of relevance to PSNA 2012 are highlighted



PSNA 2012 Program

Saturday August 11	
Time	Session Activity
12:00 – 15:00	PSNA Executive Meeting Elgin Hall Board Room
13:00 – 17:30	Registration Desk Open Elgin Hall Main Lobby
18:00 – 21:00	Evening Reception Grad Club Middlesex College

All talks and poster will be in the
Labatt Health Sciences Building (HSB)

located on Huron Rd

(See map on pp. 3)

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Sunday August 12	
Time	Session Activity
08:00	Registration Desk Open in HSB Foyer
08:15	Opening Remarks in HSB 240
08:30	Symposium I <i>Biosynthesis & Metabolism</i> Session Chair: Vincenzo De Luca
08:30 – 09:15	S1-1 ENGINEERING ELEVATED VITAMIN C IN PLANTS TO IMPROVE THEIR NUTRITIONAL CONTENT, GROWTH, AND TOLERANCE TO STRESS <u>Argelia Lorence</u>
09:15 – 10:00	S1-2 DISSECTING THE MECHANISMS, SUBSTRATE SPECIFICITIES, AND APPLICATIONS OF PLANT ENZYMES ON NATURAL PRODUCT PATHWAYS <u>Kevin D Walker</u>
10:00	Morning Refreshments
10:15	
10:30 – 11:00	S1-3 <i>Neish Young Investigator Presentation</i> RELATIONSHIPS BETWEEN THE TOXIC ALKALOID SWAINSONINE AND ENDOPHYTES <u>Daniel Cook</u> , Dale R. Gardner, Daniel Grum
11:00	S1-4 TRANSCRIPTIONAL RESPONSES TO EXOGENOUS ASPARAGINE IN ARABIDOPSIS ROOTS Sudhakar Pandurangan, Agnieszka Pajak, Ryan Austin, <u>Frédéric Marsolais</u>
11:15	S1-5 SOYBEAN <i>CHALCONE ISOMERASE</i> GENE FAMILY: CHARACTERIZATION AND ROLE IN ISOFLAVONOID BIOSYNTHESIS <u>Mehran Dastmalchi</u> , Sangeeta Dhaubhadel
11:30	S1-6 ROLE OF CAROTENOID CLEAVAGE DIOXYGENASES IN VOLATILE EMISSIONS AND INSECT RESISTANCE IN <i>ARABIDOPSIS THALIANA</i> <u>Shailu Lakshminarayan</u> , Shu Wei, Margaret Gruber, Mark A. Bernards, Lining Tian, Abdelali Hannoufa
11:45	S1-7 LIGNIN BIOSYNTHESIS IN RICE (<i>ORYZA SATIVA</i>) Taichi Koshiba, Norie Hirose, Mai Mukai, Masaomi Yamamura, Masahiro Sakamoto, Shiro Suzuki, Takefumi Hattori, <u>Toshiaki Umezawa</u>
12:00	S1-8 EFFECT OF ABA ON THE FATTY ACID ω -HYDROXYLASE 1 (FA ω H1; CYP86A33) WOUND-INDUCED EXPRESSION AND SUBERIN DEPOSITION IN SOLANUM TUBEROSUM <u>Meghan L. Haggitt</u> , Leonid V. Kurepin, Mark A. Bernards
12:15	S1-9 LOOSENING THE GRIP ON CELL WALLS: CHARACTERIZATION OF LIGNIN REGULATORY FACTORS <u>Lisa Amyot</u> , Zakir Hossain, Margaret Gruber, Brian McGarvey, Jinwook Jung, Abdelali Hannoufa
12:30	Boxed Lunch Available Young Members Meeting (HSB 240) Students, Post-docs and new faculty are encouraged to attend
12:45	
13:00	
13:15	

Contributed Talks (HSB 240) Session Chair: Mark A. Bernards	
13:30	C-1 COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI MODIFIES THE PROFILE OF BIOACTIVE PHYTOCHEMICALS IN ROOTS <u>Navid Bazghaleh</u> , Chantal Hamel, Joan Diane Knight, Yantai Gan, Andre Freire Cruz, Takaaki Ishii
13:45	C-2 IDENTIFICATION OF MOSQUITO (<i>Aedes Aegypti</i>) BITING DETERRENT FATTY ACIDS FROM THE MALE INFLORESCENCE OF BREADFRUIT (<i>ARTOCARPUS ALTILIS</i>) <u>A. Maxwell P. Jones</u> , Jerome A. Klun, Charles L. Cantrell, Diane Ragone, Kamlesh R. Chauhan, Paula N. Brown, Susan J. Murch
14:00	C-3 ANALYSIS OF BETA-METHYLAMINO-L-ALANINE (BMAA) IS COMPLEX W. Broc Glover and <u>Susan J. Murch</u>
14:15	C-4 SIX AROGENATE DEHYDRATASES SYNTHESIZE PHENYLALANINE IN <i>ARABIDOPSIS THALIANA</i> <u>Susanne E. Kohalmi</u> , Tehmina Ahmad, Crystal D. Bross, Oliver R.A. Corea, Rebecca L. Hood, Travis R. Howes, Danielle M. Styranko
14:30	C-5 COLORIMETRIC BEHAVIOR OF XYLEM SAP OBTAINED BY MECHANICAL SQUEEZE FROM SILVER BIRCH (<i>BETULA PENDULA</i>) AND ITS COLOR CONTROL <u>Akio Yamamoto</u> , Anti Rohumaa, Eero Kontturi, Mark Hughes, Pekka Saranpää, Tapani Vuorinen
14:45	C-6 PROGRAMMING AND REPRORAMMING OF GENE EXPRESSION: MECHANISMS OF CELLULAR SPECIFIC METABOLISMS? <u>De-Yu Xie</u> , Ming-Zhu Shi
15:00	C-7 NUDICAULINS, REVISED STRUCTURE AND BIOSYNTHESIS <u>Evangelos C. Tatsis</u> , Anne Christine Warskulat, Wolfgang Eisenreich, Bernd Schneider
15:15	C-8 NEW ANTILEISHMANIAL AGENTS FROM PLANTS <u>Samir A. Ross</u>
15:30 – 16:00	Break
16:00 to 19:00	Poster Session HSB Foyer Complementary Hot & Cold Hors D'oeuvres available during poster session Reception at Elgin Hall to follow

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Monday August 13	
Time	Session Activity
08:00	Registration Desk Open in HSB Foyer
08:15	
	Symposium II <i>Genomics & Bioinformatics</i> Session Chairs: Toni Kutchan and Mark Gijzen
08:30 – 09:15	S2-1 QUANTITATIVE GENOMICS OF NATURAL VARIATION IN PLANT METABOLISM: WHEN WILL THE COMPLEXITY END? <u>Daniel J. Kliebenstein</u>
09:15 – 10:00	S2-2 MAKING NEW MOLECULES <u>Anne Osbourn</u>
10:00	Morning Refreshments
10:15	
10:30	S2-3 FATTY ACID COMPOSITION OF DEVELOPING SEA BUCKTHORN (<i>HIPPOPHAE RHAMNOIDES</i> L.) BERRY AND THE TRANSCRIPTOME OF THE MATURE SEED <u>Tahira Fatima</u> , Crystal L. Snyder, William R. Schroeder, Dustin Cram, Raju Datla, David Wishart, Randall J. Weselake, Priti Krishna
10:45	S2-4 PROFILING AND STRUCTURE ELUCIDATION OF SESQUITERPENE GLYCOSIDE ISOMERS FROM WILD TOMATO SPECIES <i>SOLANUM HABROCHAITES</i> <u>E.A. Prabodha Ekanayaka</u> , A. Daniel Jones
11:00	S2-5 IDENTIFICATION AND CHARACTERIZATION OF GmMYB176 INTERACTOME IN SOYBEAN <u>Arun Kumaran Anguraj Vadivel</u> , Sangeeta Dhaubhadel
11:15	S2-6 FUNCTIONAL GENOMIC INVESTIGATION OF EPHEDRINE ALKALOID BIOSYNTHESIS IN <i>EPHEDRA SINICA</i> – CLONING AND CHARACTERIZATION OF AMINOTRANSFERASES <u>Korey G. Kilpatrick</u> , Raz Krizevski, Jillian M. Hagel, Efraim Lewinsohn, Peter J. Facchini, Frédéric Marsolais
11:30	S2-7 COMPARATIVE BIOINFORMATICS OF A HIGH AJMALICINE LINE REVEALS COMPLEX REGULATION OF MONOTERPENOID INDOLE ALKALOID BIOSYNTHESIS IN <i>CATHARANTHUS ROSEUS</i> (L.) G. DON <u>Antje M.K. Thamm</u> , Matthew Czerwinski, Ye Zhang, Kyung-Hee Kim, Christoph W. Sensen, Vincenzo De Luca
11:45-	Boxed Lunches Available Winery Tour Bus departs from the back of Elgin Hall at 12:00 Boxed Lunches should be picked up and taken on the bus. Expected return: 22:00-22:30 Afternoon free for conference participants not going on an excursion Dinner on own. (Excursions include dinner)

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Tuesday August 14	
Time	Session Activity
08:00	Registration Desk Open in HSB Foyer
	Symposium III <i>Bioproducts From Canadian Forests: Production of Valued Attributes</i> Session Chair: Mamdouh Abou-Zaid
08:30 – 08:50	S3-1 BIOPRODUCTS R&D WITHIN CANADA'S FOREST SECTOR INNOVATION SYSTEM: PLAYING A ROLE IN FOREST SECTOR TRANSFORMATION <u>Tom Rosser</u>
08:50 – 09:35	S3-2 MEDICINAL PLANTS AND PHYTOCHEMICALS FROM CANADIAN FORESTS <u>John T. Arnason</u> , Jose A. Guerrero, Andrew Waye, Jonathan Ferrier, Fida Ahmed, Carolina Cieniak, Ammar Saleem, Brendan Walshe-Roussel, Nan Shang, Steffany Bennett, Asim Muhammad, Vance Trudeau, Alain Cuerrier, Pierre Haddad
09:35 – 10:20	S3-3 PYROLYTIC CONVERSION OF BIOMASS RESIDUES INTO BIO-OIL AND BIO-CHAR: TURNING WASTE INTO BLACK GOLD <u>Franco Berruti</u>
10:20 – 10:45	Morning Refreshments
10:45 – 11:30	S3-4 ENZYME TECHNOLOGIES FOR THE PRODUCTION OF HIGH-VALUE POLYMERS AND CHEMICALS FROM FOREST RESOURCES <u>Emma R. Master</u>
11:30 – 12:15	S3-5 CONIFER SPECIALIZED DITERPENES: FROM CHEMICAL DEFENSE TO PERFUME BIOPRODUCTS <u>Philipp Zerbe</u> , <u>Jörg Bohlmann</u>
12:15	Boxed Lunch
12:30	
12:45	
13:00	
	Symposium IV (HSB 240) <i>Botanicals & Medicinals</i> Session Chair: John T. Arnason
13:15 – 14:00	S4-1 CRANBERRY: A MODEL SYSTEM FOR UNDERSTANDING PHYTOCHEMICAL COMPLEXITY <u>Paula N. Brown</u>
14:00 – 14:45	S4-2 PHYTOCHEMICALS AND METABOLIC SYNDROME <u>Ilya Raskin</u>
14:45	Afternoon Refreshment
15:15	S4-3 GENE ANALYSIS OF 4'-ACETOXY RESVERATROL: POTENTIAL HUMAN SKIN APPLICATIONS <u>Edwin D. Lephart</u> , <u>Merritt B. Andrus</u>
15:30	S4-4 FLAVONOID COMPOSITION AND EXPRESSION ANALYSIS OF FLAVONOID BIOSYNTHESIS GENES IN THE ANTIOXIDANT RICH SEA BUCKTHORN (<i>HIPPOPHAE RHAMNOIDES</i> L.) BERRIES AND LEAVES <u>Vigya Kesari</u> , <u>Tahira Fatima</u> , <u>Priti Krishna</u>

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15:45	S4-5 BLUEBERRY POLYPHENOL-ENRICHED SOYBEAN FLOUR REDUCES HYPERGLYCEMIA, BODY WEIGHT GAIN AND SERUM CHOLESTEROL IN MICE <u>Diana E. Roopchand</u> , Peter Kuhn, Leonel E. Rojo, Mary Ann Lila and Ilya Raskin
16:00	S4-6 METABOLOMICS REVEALS EFFECTS OF XANTHOHUMOL ON MARKERS OF METABOLIC SYNDROME IN ZUCKER FATTY RATS Jay Kirkwood, Leecole Legette, Cristobal L. Miranda, Yuan Jiang, Gerd Bobe, <u>Jan F. Stevens</u>
16:15	S4-7 INHIBITORY ACTIVITY OF SYNTHETIC SYRINGYL & GUAIACYL 8-O-4' NEOLIGNANS ON HUMAN CANCER CELL PROLIFERATION <u>M.S. Alam</u> , T. Katayama, T. Suzuki, M. Tokuda
16:30	S4-8 PHYTOCHEMICAL SHIKONIN CAN INDUCE IMMUNOGENIC TUMOR CELL DEATH AND ENHANCE EFFICACY OF DENDRITIC CELL-BASED CANCER VACCINE Hui-Ming Chen, Pi-Hsueh Wang, Swey-Shen Chen, Chih-Chun Wen, Yun-Hsiang Chen, Wen-Chin-Yang, <u>Ning-Sun Yang</u>
16:45	S4-9 BIOLOGICAL ACTIVITY OF LECTINS DERIVED FROM MEDICINAL PLANTS <u>Alexander V. Timoshenko</u>
17:00 – 18:00	<p>PSNA Society Member's Meeting</p> <p>All PSNA members are welcome and encouraged to attend</p> <p>Afternoon Refreshments served Member's Meeting</p> <p>(Banquet drink tickets distributed at Member's Meeting)</p>
18:00	
18:15	
18:30 – 21:30	<p>Pre-Banquet Cocktails, followed by Banquet and Awards Ceremony</p> <p>The Great Hall</p>

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Wednesday August 15	
Time	Session Activity
08:30	Symposium IV <i>Phytochemicals in the interaction between plants and their environment</i> Session Chair: Mark A. Bernards
08:30 – 09:15	S5-1 BELOWGROUND TERPENE METABOLISM: ORGANIZATION AND MULTIPLE FUNCTIONS Martha Vaughan, Qiang Wang, Jung-Hyun Huh, Reza Sohrabi, Jim Tokuhisa, <u>Dorothea Tholl</u>
09:15 – 10:00	S5-2 SQUALENE SYNTHASE AND THE ACCUMULATION OF STEROIDAL GLYCOALKALOIDS IN POTATO <u>James Tokuhisa</u> , Alice Mweetwa, Richard Veilleux, William Wadlington, Chris Wolberg
10:00	Morning Refreshments
10:15	
10:30	S5-3 <i>Neish Young Investigator Presentation</i>
10:45	ANTIFUNGAL METABOLITES FROM FUNGAL ENDOPHYTES OF <i>PINUS STROBUS</i> <u>Mark W. Sumarah</u> , Dan Sørensen, J. David Miller
11:00	S5-4 DEREPLICATION OF KNOWN PREGNANE GLYCOSIDES AND STRUCTURAL CHARACTERIZATION OF NOVEL PREGNANES IN <i>MARSDENIA TENACISSIMA</i> BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ELECTROSPRAY IONIZATION-TANDEM MASS SPECTROMETRY <u>Brian D. McGarvey</u> , Hui Liao, Keyi Ding, Xiaoling Wang
11:15	S5-5 PHYTOCHEMICAL CONTENT OF WILD ATLANTIC CANADIAN LUPINS (<i>LUPINUS POLYPHYLLUS</i> L.): OPPORTUNITIES FOR BIOPRODUCTS AND CROP DIVERSIFICATION. <u>Jason L. McCallum</u> , Chris W. Kirby, Bourlaye Fofana
11:30	S5-6 WAXES COATING THE SURFACES OF PETALS: ARE THEY DIFFERENT FROM THOSE ON VEGETATIVE ORGANS? <u>Reinhard Jetter</u> , Christopher Buschhaus
11:45	S5-7 PROGRESS TOWARDS RAPID IDENTIFICATION OF PHYTOCHEMICALS IN PLANT EXTRACTS <u>Mark A. Berhow</u> , Steven F. Vaughn, Brent Tisserat, Fred Eller
12:00	S5-8 NOVEL PYROLYSIS APPLICATIONS FOR BIOMASS CONVERSION AND NATURAL PRODUCT RECOVERY <u>Ian Scott</u> , Mohammad Hossain, Cedric Briens, Brian McGarvey, Mark W. Sumarah
12:15	Closing Remarks: HSB 240
12:30-	Boxed Lunches Available RAP Editorial Board Meeting & Incoming Executive Meeting Elgin Hall Boardroom

Invited Speaker Biographies

Argelia Lorence, PhD

Arkansas State University, Jonesboro, AR, USA



Born in Mexico City and raised in Cuernavaca, Mexico, Argelia Lorence earned her B.S. in Biochemical Engineering from the *Universidad Autónoma Metropolitana-Iztapalapa*, and her M.S. and Ph.D. in Biotechnology from UNAM, the *Universidad Nacional Autónoma de México*. She worked as an Assistant Professor at *Universidad Autónoma del Estado de Morelos* from 1998 to 2002. After doing post-doctoral training in plant metabolic engineering in the Nessler Laboratory at Texas A&M and Virginia Tech, in 2005 Lorence joined Arkansas State University (ASU) as an Assistant Professor. Currently, she is an Associate Professor with joint appointments between the Arkansas Biosciences Institute and the Department of Chemistry and Physics.

Lorence was part of the group that at Virginia Tech discovered a new route leading to vitamin C formation in plants. This pathway involves four enzymes in the conversion of *myo*-inositol to vitamin C (a.k.a. ascorbic acid, AsA). Her group at ASU has engineered plants to contain elevated levels of this essential vitamin and demonstrated that in addition to having enhanced nutritional content, these plants grow better, accumulate more biomass and become tolerant to multiple stresses including heat, soil salinity, herbicides, and environmental pollutants. In addition to *Arabidopsis* (*Arabidopsis thaliana*), her models of study include rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and tobacco (*Nicotiana tabacum* and *Nicotiana benthamiana*).

Since joining ASU Lorence has secured over 3.5 million dollars in grants, co-authored 20 publications, and given 150 presentations at regional, national and international meetings. In 2004 she co-edited the book “Recombinant Gene Expression: Reviews and Protocols, Second Edition” that became a best-seller for Humana Press (recently acquired by Springer). The third edition of this book of which she is sole editor became available in January 2012.

Among the awards she has received are the *Gabino Barreda* and *Alfonso Caso* Medals from UNAM, the *Arthur Neish Young Investigator Award* from the Phytochemical Society of North America, the nomination as *Featured Mentor* from the Minority Environmental Leadership Development Initiative of the University of Michigan, the *Dean Horizons Award* from ASU, and being nominated to join the Agriculture and Biotechnology section of *Faculty of 1000*, a premier post-publication peer review system led by a faculty of 5,000 of the world’s leading scientists and clinical researchers. As part of the celebrations of the International Women’s Day, in 2010 Lorence received the *Distinguished Woman in Science Award* from the Congress of the State of Morelos. In addition, the government of the city of Cuernavaca, where Dr. Lorence worked and lived for many years, also gave her a special award for her contributions to science and technology in Morelos. In 2011 she received the *Outstanding Hispanic Achiever of the Year Award* from the Hispanic Community Services of Jonesboro, AR.

Kevin Walker, PhD

Chemistry/Biochemistry & Molecular Biology
Michigan State University



Kevin Walker earned his BS in Chemistry in June 1988 at the University of Washington, Seattle. Thereafter, he worked at the Seattle District Laboratory, Food and Drug Administration where he worked with senior scientists to develop various analytical techniques that identified xenobiotic compounds in aquatic species from June 1988 to August 1990. He then earned a PhD in Organic Chemistry in 1997 working on various natural product biosynthetic pathways in the laboratory of Prof. Heinz Floss at the University of Washington. He continued his training in Chemistry/ Biochemistry and Molecular Biology at Washington State University (Institute of Biological Chemistry) where he was an NIH Postdoctoral researcher with Prof. Rodney Croteau from 1997-1999. He continued at the institute as a Research Assistant Scientist until December 2003. In January 2004, he became an Assistant Professor at Michigan State University, serving in the Departments of Chemistry and Biochemistry and Molecular Biology. He is currently an Associate Professor at MSU since 2010 where he is investigating the biocatalysis of bioactive natural products and investigating enzyme mechanisms.

Anne Osbourn, PhD

John Innes Centre, Norwich Research Park, UK



Anne Osbourn is a Project Leader in the Department of Metabolic Biology at the John Innes Centre. She also leads the Institute Strategic Programme on Plant and Microbial Metabolism and is an Associate Research Director of the Centre. Her research focuses on plant natural products - function, synthesis and metabolic diversification. Anne's group works with crop and model plants, and uses a wide range of multidisciplinary approaches including genetics, genomics, computational biology, cell biology, protein and small molecule biochemistry. Anne is an author of over 100 peer-reviewed scientific publications and recently co-edited a comprehensive textbook on plant-derived natural products [Lanzotti V & Osbourn A. (2009) Plant-derived natural products – Synthesis, function and application. Springer, New York, USA]. She has also developed and co-ordinates the Science, Art and Writing (SAW) initiative, a cross-curricular science education programme for schools (www.sawtrust.org).

Daniel Kliebenstein, PhD

Department of Plant Sciences, University of California Davis



Dr. Kliebenstein obtained his bachelors degree at Iowa State University, his doctorate at Cornell University and did a postdoctoral research program at the Max Planck institute for Chemical Ecology in Jena, Germany. All three steps of his career path had a dual focus on biochemistry and genetics in all forms. This diverse background has allowed Dr. Kliebenstein to study plant biochemical genetics and use this work to begin answering questions in two previously distinct research areas.

The first research area is plant biochemistry and its regulation by focusing on how and why plants make secondary metabolites plant compounds that provide the taste, flavor, color and medicinal activities that people associate with specific plants. However, their primary role appears to be helping the plant cope with its environment by attracting pollinators, repelling attackers and protecting the plant from sunlight. This research avenue has allowed Dr. Kliebenstein to make significant contributions to the study of systems biology and how to properly integrate different genomics datasets into a single analysis. These novel systems approaches are allowing Dr. Kliebenstein to show that there are likely several thousand genes controlling the production of the glucosinolate secondary metabolites and more novel, that the secondary metabolites themselves appear to have the ability to alter key physiological processes in the plant such as the circadian clock. This raises the question of how secondary are these compounds.

The second major research area is quantitative and population genomics where Dr. Kliebenstein uses the same information to understand how and why organisms have genetic variation in adaptive phenotypes such as secondary metabolites. To ask this question, he utilizes the same secondary metabolites as phenotypes to study and develop methodology to understand the underpinnings of quantitative genetics and genomics. This systems approach has allowed him to develop novel network based algorithms to rapidly identify causal genes in both QTL mapping and genome wide association approaches that can be used in any species. This is showing that adaptive phenotypes are controlled by hundreds to thousands of genes. Further, there are significant negative pleiotropic interactions between phenotypes such that polymorphisms in plant defense metabolism can impact growth and flowering of the plant via likely direct mechanistic interactions. Together these two questions are allowing him to make significant progress to a systematic understanding of plant defense metabolism biology.

Tom Rosser, MSc

Assistant Deputy Minister, Canadian Forest Centre, Natural Resources Canada, Ottawa, ON



Tom Rosser is the Assistant Deputy Minister of the Canadian Forest Service (CFS) of Natural Resources Canada.

Prior to taking on these responsibilities, Tom served as Director General of the Policy, Economics and Industry Branch of the CFS. In this position, he was responsible for leading the trade and international affairs, economics and policy development functions of the organization. He was also responsible for a number of programs that promote market development and innovation in the Canadian forest sector as well as improved sustainability of the Canadian pulp and paper industry.

Earlier in his career, Tom held a number of positions in both the public and private sectors related to economic and public policy analysis in natural resource sectors. This includes assignments at Natural Resources Canada, Industry Canada and the Forest Products Association of Canada.

A British Chevening Scholar, Tom holds a M.Sc. in Environmental and Resource Economics from the University of London as well as Masters and Bachelors degrees in Public Administration from Carleton University in Ottawa.

Natural Resources Canada is a federal government department that includes responsibilities for the energy and energy technology, minerals and metals, Earth sciences and forest sectors. The Canadian Forest Service is a science-based policy organization with close to 1,000 employees working in forest research centres in five locations across Canada, as well as in science and economic policy coordination functions in Ottawa.

John Thor Arnason, PhD

Department of Biology, University of Ottawa



John Thor Arnason is Professor of Biology at the University of Ottawa and Director of the Biopharmaceutical Sciences program. Arnason's laboratory specializes in the phytochemistry and biological activity of medicinal plants. His lab has studied unique North American germplasm such as Ontario ginseng, Nunavik Rhodiola and boreal Vacciniums. As part of a team grant on aboriginal medicine, his group has undertaken studies on the efficacy and safety of Northern Cree traditional medicines used for type 2 diabetes. The laboratory has also studied the ethnobotany and ethnopharmacology of Maya medicinal plants focusing on plants used for anxiety, epilepsy and dementia. He has served as an advisor on the scientific board of natural health product companies, American

Botanical Council and Health Canada's expert advisory board on Natural Health Products.

Emma R. Master PhD

Department of Chemical Engineering and Applied Chemistry, University of Toronto



Emma Master earned a B.Sc. in Microbiology from McGill University in 1995, and a Ph.D. in Environmental Biochemistry from UBC in 2002. In 2002, she joined the Royal Institute of Technology in Stockholm, Sweden (KTH) as a Post-doctoral Fellow. There, she studied wood fibre biosynthesis and modification by carbohydrate-active enzymes and led the enzyme discovery and characterization group of KTH's wood biotechnology division. In 2004, she joined the Fungal Genomics Project at Concordia University, before she became faculty member in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto in 2005. The overall aim of Emma's research program is to design and produce biological catalysts that synthetically derivatize or modify biopolymers and chemicals derived from plants. In this way, her group is developing biotechnologies to produce novel, high-performance biomaterials and

specialized biochemicals that provide renewable alternatives to petroleum-based compounds. Emma's research program is motivated by 1) the importance of value-added products from lignocellulosic biomass to the diversification of agricultural and forest products and invigoration of corresponding industries in Canada, 2) the role of high-value co-products from biomass in lowering the cost of biofuel production in the short term, while providing long-term security for investments in lignocellulose biorefineries, and 3) the recognition that biological systems sustainably synthesize the most diverse and specialized biomaterials and chemicals known, most of which have gone untapped. Emma received a Finish Distinguished Fellowship (FiDiPro) in 2010 and an Early Researcher Award from the Ontario Ministry of Research and Innovation in 2009. She is a theme leader in the NSERC Bioconversion Network, and the Genome Canada project "BEEM"; she also leads "Forest FAB: Applied Genomics for Functionalized Fibre and Biochemicals", a 4-year collaborative research program funded by the Ontario Ministry for Economic Development and Innovation.

Philipp Zerbe, PhD

University of British Columbia, Michael Smith Laboratories



Philipp Zerbe is a Research Associate with Jörg Bohlmann in the Michael Smith Laboratories at the University of British Columbia, Vancouver, Canada. He received his PhD from the Ruhr University Bochum, Germany (2007) under mentorship of Professor Elmar Weiler, focussing on the structural and functional interrelations of key enzymes in jasmonate biosynthesis. In Prof. Bohlmann's group he is leading research on the elucidation and engineering of plant specialized diterpene biosynthetic pathways as part of two Genome Canada funded projects (SMarTForests, PhytoMetaSyn) and an ongoing NSERC

funded program on conifer terpenoids. His research interests focus on the evolutionary diversification of terpenoid biosynthesis in various medicinal and other commercially relevant plants and the development of tools for the production of terpenoid metabolites with human benefit.

Franco Berruti, PhD

Department of Chemical and Biochemical Engineering, Western University, London, ON



In 1982, after graduating from Chemical Engineering at the Politecnico of Torino in Italy, and being conferred the designation of Dott. Ing., Franco Berruti immigrated to Canada. Here he completed, at the University of Waterloo, a MSc (1983) and a Ph.D. (1986), both in Chemical Engineering. Dr. Berruti has dedicated his career towards the discovery of innovative, chemical engineering processes and technologies, designed to meet industrial and societal needs. He has applied chemical reactor and fluidized bed technologies for transforming heavy oils, biomass or residue materials into valuable chemicals or environmentally friendly fuels. Franco Berruti has strongly advocated the education of big-picture engineers. As Associate Dean at the University of Calgary and Dean of the University of Saskatchewan's College of Engineering and then at

The University of Western Ontario's Faculty of Engineering (Western Engineering), Franco Berruti took the leadership opportunities to build strong ties with industry and community. At Western Engineering, he created an entrepreneurial certificate for undergraduate students, and strongly promoted concurrent degrees for engineering students, such as graduation with degrees in engineering and science or engineering and business. He played a lead role in the introduction of Green Process Engineering, a first of its kind program in Canada, to Western Engineering. In 2008, Franco Berruti stepped down as Dean of Engineering to create, with his primary research collaborator, Professor Cedric Briens, a new 20,000 sq.ft. University of Western Ontario institute, ICFAR, the Institute for Chemicals and Fuels from Alternative Resources. From ICFAR, Franco Berruti has led an \$8.7 M research network funded by Agriculture and Agri-Food Canada, ABIN (Agricultural Biorefinery Innovation Network for Green Energy, Fuels and Chemicals), involving industry, government and academia. He is presently a Platform Lead of BioFuelNet, a recently announced Canadian network with close to 100 researchers that will receive almost \$25 M from Canada's Networks of Centres of Excellence (NCE) program.

Dr. Berruti's approach is to develop strong collaborations, where the research team considers not just the problem at hand, but related factors including environmental issues such as sustainability, efficiencies, and, economy. Because of this, he and his collaborators have been extremely successful in integrating academic research with industrial application. In terms of technology transfer and commercialization, Franco Berruti holds 4 patents, and has 2 patents pending. A spin-off company, Agri-Therm Inc., was created in London, Ontario, commercializing the energy-efficient, mobile pyrolysis equipment used to process biomass into useful bio-products, that Franco Berruti developed together with Dr. C. Briens. Professor Berruti is the senior founding co-editor of the International Journal of Chemical Reactor Engineering (IJCRE). To date, he has supervised/co-supervised over 80 graduate students and 23 postdoctoral fellows and research engineers. He has over 300 publications. Over the past 10 years, he has been awarded more than \$15.5 M in research funding, with about \$3 M through industrial partnership. Professor Berruti has co-chaired 9 International Conferences and is extensively involved in international collaborative projects in the USA, UK, Brazil, Mexico, France, Italy, Germany, Spain, and India. He serves on numerous committees and boards, including the Mayor's Sustainable Energy Council, in London Ontario. In 2011, Franco Berruti was honoured with the 2011 Ontario Green Chemistry and Engineering Award.

Ilya Raskin, PhD

Global Institute for BioExploration, Rutgers University



Ilya Raskin, Ph.D. has over 25 years of experience in academic research in plant biology and pharmacology and 5 years of experience in industrial research in plant biotechnology at DuPont Co. Dr. Raskin received a Ph.D. from Michigan State University in 1984 and joined the faculty of Rutgers, The State University of New Jersey, in 1989 where he currently works as Professor II and President of the Global Institute for Bio-Exploration (GIBEX). Dr. Raskin is also a member of the NIH Center for Botanicals and Metabolic Syndrome.

Dr. Raskin earned an international reputation through his work on plant growth regulation, plant immunity to diseases, phytoremediation, and, in the last decade, for his research in plant-derived foods and medicines. He is also actively involved in international bioexploration and conservation. Dr. Raskin's research is featured in over 170 major scientific publications and in numerous popular press articles. He has been listed as one of 108 most cited researchers in Plant and Animal Science (<http://isihighlycited.com> Institute of Scientific Information). Among his most cited scientific publications are four cover articles in *Science* and *Nature*. Dr. Raskin was also awarded 20 patents covering the discoveries made in his laboratory. Dr. Raskin has received a number of prestigious awards, including the Albert Shull Award for outstanding contributions to plant biology and the Thomas Alva Edison Patent Awards for revolutionary product innovation and scientific breakthrough. Dr. Raskin was named the Century Innovator in Botany by the U.S. News & World Report.

Paula Brown, PhD

BC Institute of Technology, Vancouver, BC



Dr. Paula Brown completed a B.Sc. Honours in Chemistry and Biochemistry at Dalhousie University (1996), her M.Sc. in carbohydrate synthesis at Simon Fraser University (1998) and received her PhD from the University of British Columbia (2011) conducting studies on plant metabolomics. Through her position at the BC Institute of Technology she has supported the natural health and food product industry for more than a decade by conducting applied research on product quality, safety and efficacy. Dr. Brown was appointed Fellow of the AOAC in 2009 having served five years as General Referee for the Dietary Supplements Committee, seven on the Dietary Supplement Task Force, participating on eight Expert Review Panels, and directing three collaborative studies. She is currently serving her third term on the Investment Agriculture

Foundation of BC Board of Directors, her second term on the Natural Health Products Program Advisory Committee for Health Canada, and serves on review panels for the National Center for Complementary & Alternative Medicine, NIH. Dr. Brown is Chair of NSF's Joint Committee for Dietary Supplements and an Advisory Board member for the American Botanical Council. She is the Director of the Natural Health & Food Products Research Group at the British Columbia Institute of Technology and the Quality Focus columnist for *Nutraceuticals World*.

Dorothea Tholl, PhD

Department of Biological Sciences, Virginia Polytechnic Institute



Dorothea Tholl is an Associate Professor in the Department of Biological Sciences at Virginia Tech. Dr. Tholl received her Ph.D. in Pharmaceutical Biology (1996) with Honors from the Technical University of Braunschweig, Germany. From 1997-2003 she was a postdoctoral fellow and junior group leader with Jonathan Gershenzon at the Max Planck Institute for Chemical Ecology, Germany. She held a joint appointment with the MPI for Chemical Ecology and the University of Michigan from 2003-2005 before she became Assistant Professor in Biological Sciences at Virginia Tech. In 2007, she received the PSNA Arthur C. Neish Young Investigator Award. She currently serves on the advisory board of “New Phytologist” and on the editorial board of “The Plant Journal”. Dr. Tholl’s research interests include the

biochemistry and chemical ecology of plant volatiles. Her recent studies focus on the plasticity and organization-function relationships of plant specialized metabolism above- and below-ground.

Jim Tokuhsa, PhD

Department of Horticulture, Virginia Polytechnic Institute



James G. Tokuhsa is an Assistant Professor in the Department of Horticulture at Virginia Tech working on plant chemical defenses in *Arabidopsis* and a wild relative of potato. His research career has focused on plant adaptation to the environment. He received a B.S. in Botany with Distinction from the University of Illinois-Urbana having completed undergraduate research in the laboratory of Larry Vanderhoef on high affinity auxin binding activity in soybean hypocotyl extracts. He earned his Ph.D. in Botany under the guidance of Peter Quail at the University of

Wisconsin-Madison in 1986 characterizing a novel phytochrome photoreceptor in light-grown *Avena sativa*, the second receptor of what is now recognized as a large family in higher plants. He joined the research group of Jim Peacock and Liz Dennis at the CSIRO Division of Plant Industry in Canberra to investigate promoter element sequences required for protein binding and transcriptional activation of genes associated with *Agrobacterium* infection of tobacco. These studies were followed by research with John Browse at Washington State University to identify genes and their functions in plant chilling resistance using *Arabidopsis* T-DNA tagged mutants. He worked at the Max Planck Institute for Chemical Ecology, mentored by Jonathan Gershenzon, to characterize enzymes in the biosynthesis of glucosinolates and insect behavior feeding on mutant *Arabidopsis* lines. He was hired at Virginia Tech in 2005, and has continued research on the glucosinolate-myrosinase system of *Arabidopsis* and developed projects on the biosynthesis of steroidal glycoalkaloids in *Solanum chacoense* and the graft transmission of stress tolerance in tomato. He teaches an undergraduate course titled “Environmental Factors in Horticulture” with a theme of sustainability and emphasis on agroecology.

Neish Young Investigator Biographies

Daniel Cook, PhD

USDA-ARS-NPA Poisonous Plant Research, Logan UT, USA



Daniel Cook is a research scientist at the USDA ARS Poisonous Plant Research Laboratory in Logan, UT. His primary research interest is describing the relationship between swainsonine containing plants and fungal endophytes. Additionally he pursues research describing the chemical ecology of plant toxins focusing two other genera of plants and their toxins: *Delphinium* (norditerpene alkaloids) and *Lupinus* (quinolizidine and piperidine alkaloids). The influence of environment, development, and genetics on toxin concentrations, synthesis, and subsequent risk of poisoning are being investigated to mitigate livestock losses. Previous to his current position, Daniel was a post-doctoral associate at the USDA ARS Natural Products Utilization Research Unit in Oxford, MS where he identified and characterized a novel polyketide synthase that uses a fatty acyl-CoA as a substrate to form an alkylresorcinol. Daniel earned a Ph.D. from Michigan State University in Plant Biology where he studied the process of cold acclimation in *Arabidopsis thaliana* in the laboratory of Dr. Mike Thomashow. Daniel earned a B.S. from Utah State University in Crop Science.

Mark Sumarah, PhD

Southern Crop Protection Food Research Centre, Agriculture and Agri-Food Canada, London, ON, Canada



Mark Sumarah was born and raised in Halifax, Nova Scotia. He received his undergraduate degree at Saint Mary's University in Halifax, and then completed his MSc and PhD in Chemistry at Carleton University in Ottawa, Ontario with Professor J. David Miller. Dr. Sumarah held a joint post-doctoral position with Carleton University and the Merck Frosst Centre for Therapeutic Research in Montreal, Quebec. He currently works as a Research Scientist with Agriculture and Agri-Food Canada in London, Ontario at the Southern Crop Protection Food Research Centre. His expertise is in the isolation, structural elucidation and analysis of small organic molecules from complex biological matrices using LC-MS and NMR. Dr. Sumarah's current research is focused on the use of metabolomics to study the mechanism of resistance to disease in Canadian crops. He has authored or co-authored 15 publications, and owns a patent.

Symposium I Biosynthesis & Metabolism

S1-1

ENGINEERING ELEVATED VITAMIN C IN PLANTS TO IMPROVE THEIR NUTRITIONAL CONTENT, GROWTH, AND TOLERANCE TO STRESS

Argelia Lorence^{1,2}

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Vitamin C (L-ascorbic acid, AsA) is essential to both plants and animals. Humans are incapable of synthesizing this vitamin and therefore must acquire it from fresh fruits and vegetables. As a result, there is considerable interest in enhancing AsA content in a wide variety of food crops. Four AsA biosynthetic pathways are known to operate in plants involving L-galactose, D-glucuronate, L-gulose, and *myo*-inositol as main precursors. Work in my group focuses on understanding the contribution of the inositol pathway to AsA and the regulation of this complex metabolic network. In this presentation I will discuss our progress on the characterization of *myo*-inositol oxygenase (MIOX), glucuronate reductase (GlcUR), gluconolactonase (GNL), and L-gulonolactone oxidase (GLOase), the enzymes catalyzing the conversion of free inositol into AsA. In *Arabidopsis* over-expression of MIOX and GLOase leads to plants with enhanced growth, biomass, and tolerance to a wide range of abiotic stresses including salt, heat, cold, herbicides, and environmental pollutants. We have also been successful at engineering high AsA in tobacco and rice after constitutive expression of MIOX and GLOase. Currently we are evaluating the effects of this modification on the growth of the over-expressers and also in their ability to interact with foliage and sap-eaters.

S1-2

DISSECTING THE MECHANISMS, SUBSTRATE SPECIFICITIES, AND APPLICATIONS OF PLANT ENZYMES ON NATURAL PRODUCT PATHWAYS

Kevin D Walker

Chemistry/Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI 48824-1322

In *Taxus* plants, the biosynthesis of the pharmaceutical paclitaxel relies, in part, on the transfer of β -amino phenylpropanoids from coenzyme A to the terpenoid baccatin III by an acyl CoA-dependent acyltransferase. Several enzymes on the pathway are known, yet a few remain unidentified, including the putative ligase that biosynthesizes the key β -amino phenylpropanoyl CoA thioesters. The multienzyme, nonribosomal peptide synthetase that produces tyrocidines A-D contains a tridomain starter module tyrocidine synthetase A (TycA). TycA normally activates (*S*)- α -phenylalanine to an adenylate anhydride and transfers the amino acid moiety to a pendent pantetheine of the adjacent thiolation (T) domain. The adenylation domain of TycA was found to function as an amino phenylpropanoate:CoA ligase and make CoA thioesters that are substrates of the phenylpropanoyltransferase (PPT) on the paclitaxel biosynthetic pathway. The PPT enzyme uses (*R*)- β -phenylalanine as one of its substrates, which is derived from (*S*)- α -phenylalanine *via* a *Taxus* phenylalanine aminomutase. Aspects of the TycA "CoA ligase" reaction and the aminomutase stereochemistry and mechanism are described.

S1-3

RELATIONSHIPS BETWEEN THE TOXIC ALKALOID SWAINSONINE AND ENDOPHYTES

Daniel Cook¹, Dale R. Gardner¹, Daniel Grum¹

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Locoweeds are *Astragalus* and *Oxytropis* species that contain the trihydroxyindolizidine alkaloid, swainsonine. A fungal endophyte, *Undifilum oxytropis* found in locoweed plant species is responsible for the synthesis of swainsonine. Two chemotypes of *Oxytropis* and *Astragalus* locoweeds were identified from field collections, plants that accumulate high concentrations of swainsonine and plants that accumulate low or non-detectable swainsonine concentrations. The plants with high swainsonine concentrations had relatively high amounts of endophyte while plants with low or non-detectable swainsonine concentrations had low endophyte amounts. Data will be presented characterizing the relationship between swainsonine and the endophyte in these two chemotypes. Additionally, other plant species have been documented to contain swainsonine, including some *Swainsona* (Leguminosae) species in Australia and some *Ipomoea* (Convolvulaceae), *Turbina* (Convolvulaceae), and *Sida* (Malvaceae) species in South America and Africa. Fungal endophytes that produce swainsonine have been isolated from *Swainsona canescens* and *Ipomoea carnea*. Data will be presented characterizing these novel endophyte species.

S1-4

TRANSCRIPTIONAL RESPONSES TO EXOGENOUS ASPARAGINE IN ARABIDOPSIS ROOTS

Sudhakar Pandurangan^{1,2}, Agnieszka Pajak², Ryan Austin², Frédéric Marsolais^{1,2}

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²Genomics and Biotechnology, Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON, Canada N5V 4T3

Asparagine is a major form of nitrogen storage and transport in higher plants. This amide amino acid can sustain Arabidopsis growth as a single nitrogen source. However, at the seedling stage, it inhibits root elongation and root hair formation in a dose-dependent manner, relative to control conditions without nitrogen. These responses are related to the amount of internal asparagine, as demonstrated in studies performed with asparaginase deficient mutants. To investigate transcriptional responses to asparagine, Arabidopsis seedlings were grown for ten days and transferred to media with or without 20 mM asparagine for two hours. Under these conditions, internal asparagine concentration in roots was raised by five-fold. RNA was extracted from quadruplicate samples and transcripts profiled by Illumina Gaiix sequencing. Paired-end, 100 base pair reads were mapped to Arabidopsis gene models. A total of 95 genes had transcript levels elevated by more than two-fold, with 12 were decreased by more than two-fold, at a false discovery rate less than 0.001. These include several genes related to nitrogen transport, metabolism and storage. The results provide information on transcriptional responses elicited by asparagine in root, and identify several marker genes which could be used to further investigate the signal transduction pathway leading to these responses.

S1-5

SOYBEAN *CHALCONE ISOMERASE* GENE FAMILY: CHARACTERIZATION AND ROLE IN ISOFLAVONOID BIOSYNTHESIS

Mehran Dastmalchi^{1,2}, Sangeeta Dhaubhadel^{1,2}

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Isoflavonoids are plant secondary metabolites produced via a legume-specific branch of the phenylpropanoid pathway. They are actors in signaling for nitrogen fixation and plant response to stress. Isoflavonoids are noted for their human health benefits. Chalcone Isomerase (CHI) catalyzes the reaction producing flavanones, the skeletal backbone for isoflavonoids. There are eight *CHI* genes in the soybean genome including the novel *CHI3B*. We identified *CHI3B* through *in silico* analysis and confirmed expression in roots of four soybean cultivars. *CHI3B* has high levels of similarity, at the amino acid level, with *CHI3A*. *CHI* gene family members showed different temporal and spatial expression in soybean tissue. At a subcellular protein level, CHI isozymes with the catalytic capability to produce flavanones were localized to the nucleus and cytoplasm. Quantitative gene expression analysis of the *CHI* family, in soybean roots, with different isoflavonoid levels, showed *CHI2* expression corresponds with higher isoflavonoid content. The function of *CHI2 in planta* has been further studied through silencing of *CHI2* using soybean hairy root transformation, and subsequent analysis of isoflavonoid levels. Our results suggest that *CHI2* is the isoflavonoid-specific member of the family. Identification of factors regulating CHI will help our understanding of the genetic and molecular basis of isoflavonoid biosynthesis.

S1-6

ROLE OF CAROTENOID CLEAVAGE DIOXYGENASES IN VOLATILE EMISSIONS AND INSECT RESISTANCE IN *ARABIDOPSIS THALIANA*

Shailu Lakshminarayan^{1,2}, Shu Wei³, Margaret Gruber³, Mark Bernards², Lining Tian^{1,2}, and Abdelali Hannoufa^{1,2}

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Carotenoid degradation by enzymatic oxidative cleavage produces an array of terpenoid products that are collectively known as apocarotenoids, which include volatile and non-volatile compounds. Previous studies showed that volatile apocarotenoids have a deterring effect on insects. In *Arabidopsis thaliana*, a family of nine genes known as carotenoid cleavage dioxygenases are involved in apocarotenoid production in plants. Of these, four encode carotenoid cleavage dioxygenases (*CCDs* – *CCD1*, *CCD4*, *CCD7*, and *CCD8*), and five encode 9-*cis*-epoxycarotenoid dioxygenases (*NCEDs* – *NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9*). A previous study involving *CCD1* overexpression in *Arabidopsis* revealed that transgenic *Arabidopsis* plants had significantly enhanced β -ionone emissions, which resulted in a significant decrease in insect feeding damage relative to wild type control. On the basis of this study, we have initiated a study to investigate the effects of overexpression of the other eight members of the *CCD/NCED* gene family on volatile apocarotenoid emissions and on insect-plant interactions. We will present our previous work involving overexpression of *CCD1* gene, and will provide an update on our current research.

S1-7

LIGNIN BIOSYNTHESIS IN RICE (*ORYZA SATIVA*)

Taichi Koshiba¹, Norie Hirose¹, Mai Mukai¹, Masaomi Yamamura¹, Masahiro Sakamoto², Shiro Suzuki¹, Takefumi Hattori¹, Toshiaki Umezawa^{1,3,*}

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We have analyzed the physiological function of the rice (*Oryza sativa* L. cv. Nipponbare) OMT annotated as caffeic acid *O*-methyltransferase 3 (OsCAOMT3). Recombinant CAOMT3 catalyzed the 5-methylation of 5-hydroxyferulate and 5-hydroxyconiferaldehyde, and the methylation of 5-hydroxyferulate was inhibited by 5-hydroxyconiferaldehyde. The rice plant which was down-regulated in expression of *OsCAOMT3* exhibited a weakened staining with Wiesner reagent in the walls of vascular bundle cells and sclerenchyma tissue compared to wild-type plant. Lignin content of the transgenic shoot decreased, and its syringyl lignin content was reduced up to 87% than that of wild-type. In addition, we prepared transgenic rice plants in which expression of genes encoding *p*-coumaroyl shikimate/quinic acid 3-hydroxylase (C3'H) and hydroxycinnamoyl CoA: shikimate/quinic acid hydroxycinnamoyl transferase (HCT) were down-regulated individually. Lignin contents of both transgenic plants were lower than that of wild-type plant. Taken together, these data indicate that the physiological function of OsCAOMT3 is to catalyze the 5-methylation in the syringyl lignin biosynthesis as 5-hydroxyconiferaldehyde OMT (CAldOMT). The data also suggest that the biosynthetic pathway of syringyl lignin in rice is similar to those in dicotyledonous plants.

S1-8

EFFECT OF ABA ON THE FATTY ACID ω -HYDROXYLASE 1 (FA ω H1; CYP86A33) WOUND-INDUCED EXPRESSION AND SUBERIN DEPOSITION IN SOLANUM TUBEROSUM

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Suberin is a complex macromolecule deposited in response to wounding to prevent desiccation and pathogen infection, and comprises of two distinct but covalently-linked domains. One domain is polyphenolic in nature, assembled from hydroxycinnamic acids, hydroxycinnamoyl amides and monolignols; while the other is polyaliphatic in nature, assembled primarily from fatty acids, ω -hydroxy fatty acids, α,ω -dioic fatty acids and glycerol. In *Solanum tuberosum*, over 55% of the fatty acids incorporated into the aliphatic domain of suberin are either ω -hydroxy fatty acids or α,ω -dioic fatty acids, having been modified by a fatty acid ω -hydroxylase. Potato Fatty Acid ω -Hydroxylase 1 (*StFA ω H1*; CYP86A33) is highly expressed in suberizing tissue and its homolog from *Arabidopsis* CYP86A1 has been shown to catalyze the ω -hydroxylation of fatty acids. Functional characterization by expression of recombinant protein is in progress for *StFA ω H1*. *In silico StFA ω H1* promoter analysis identified eleven abscisic acid (ABA)-like response elements, suggesting a regulatory role for ABA in aliphatic suberin biosynthesis. Treatment of potato tuber tissue with fluoridone (an inhibitor of endogenous ABA biosynthesis) and exogenous ABA showed that *StFA ω H1* gene expression is accelerated in the presence of ABA, indicating an ABA-dependent effect on transcription. Further characterization

of suberin quantity and composition is in progress to determine the effect of ABA on suberin monomer deposition. In addition, *Agrobacterium* infiltration of *Nicotiana benthamiana* leaves with twelve promoter deletion constructs based on the removal of ABA-like response elements are being tested for their ability to drive wound-inducible β -glucuronidase expression. An in-depth promoter study and comprehensive suberin analysis will provide a better understanding of the regulation acting on this key enzyme in suberin biosynthesis, which in future could be used as a proxy for understanding aliphatic suberin regulation as a whole.

S1-9

LOOSENING THE GRIP ON CELL WALLS: CHARACTERIZATION OF LIGNIN REGULATORY FACTORS.

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Brassica napus (canola) and *Medicago sativa* (alfalfa) are target crops for biofuel production. Our goal is to optimize their cell walls to make them more amenable to cellulosic-biofuel processes by modifying their lignin content and composition. Using microarray and expression analyses, we identified a number of genes in *B. napus* and *M. sativa* that were differentially regulated in low vs. high lignin material. We fully characterized three of these genes in the model plant, *Arabidopsis*. *DIMINUTO1*, a brassinosteroid biosynthetic gene, and *EF1B β* , a guanine nucleotide exchange factor, were both shown to affect vascular development and xylem vessel formation in the inflorescence stem. We also demonstrated that *HB5*, an ABA-responsive HD-bZIP transcription factor, is down-regulated in *Arabidopsis* mutants with knockouts in the genes *NST1*, *VND7*, and *MYB46*, whose products are key regulators of lignin biosynthesis. Histochemical staining as well as lignin content and monomeric analyses validated that all three of these genes affect lignin content and composition as well as cell wall structure. These genes are thus good candidates to deploy in major biofuel crops, including canola and alfalfa.

Contributed Paper Session

C-1

COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI MODIFIES THE PROFILE OF BIOACTIVE PHYTOCHEMICALS IN ROOTS

Navid Bazghaleh^{1,2}, Chantal Hamel^{1,2}, Joan Diane Knight², Yantai Gan¹, Andre Freire Cruz³, Takaaki Ishii³

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The structure and function of microbial communities in the rhizosphere are continuously modified through the release of a wide range of bioactive phytochemicals. The effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on the production of bioactive phytochemicals was investigated within roots of chickpea (*Cicer arietinum* L.) cultivar CDC Anna. Root proteins and compounds soluble in methanol were extracted from mycorrhizal and non-mycorrhizal roots and fractionated using HPLC. The fractions were recovered and their bioactivities on two endophytic fungi (*Trichoderma harzianum* and *Geomyces vinaceus*) and two pathogenic fungi (*Fusarium oxysporum* and *Rhizoctonia* sp.) were assayed in microtitre plates. A 24 kDa protein fraction over expressed in mycorrhizal roots inhibited the growth of the endophytic and pathogenic fungi. Furthermore, the 25%-MeOH extract from the mycorrhizal roots had 9 bioactive fractions compared to 18 fractions from the non-mycorrhizal roots. Several active fractions stimulated the growth of *Trichoderma harzianum* and *Geomyces vinaceus*, and some inhibited *Rhizoctonia* sp. and *Fusarium oxysporum*. Non-protein phytochemicals had selective effects on the endophytes and pathogens whereas the antifungal proteins of mycorrhizal root were non-selective.

C-2

Identification of Mosquito (*Aedes aegypti*) Biting Deterrent Fatty Acids from the Male Inflorescence of Breadfruit (*Artocarpus altilis*)

A. Maxwell P. Jones¹, Jerome A. Klun², Charles L. Cantrell³, Diane Ragone⁴, Kamlesh R. Chauhan², Paula N. Brown^{5,6}, and Susan J. Murch⁶

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Breadfruit (*Artocarpus altilis*) is a high yielding staple crop cultivated throughout the wet tropics. The dried male inflorescence of this tree is traditionally used as a smudge in communities throughout Oceania to repel flying insects, including mosquitoes. This study was conducted to evaluate this practice and identify the chemicals responsible for mosquito deterrence. Various crude extracts were evaluated in a preliminary screen, and the most active extract, the hydrodistillate, was selected for bioassay-guided fractionation. The hydrodistillate and all fractions displayed significant deterrent activity to the *Aedes aegypti* mosquito. Exploratory GC-MS analysis revealed more than 100 distinctive peaks and more than 30 compounds were putatively identified, and included a mixture of terpenes, aldehydes, fatty acids,

and aromatics. A systematic bioassay-directed study using adult *Aedes aegypti* females identified capric, undecanoic, and lauric acid as primary deterrent constituents. A synthetic mixture of fatty acids that were present in the most active fraction, as well as the individual fatty acids were all significantly more deterrent than N,N-diethyl-m-toluamide (DEET). These results provide evidence in support of this traditional practice and indicate the potential of male breadfruit flowers and fatty acids as natural sources of mosquito repellents.

C-3

ANALYSIS OF BETA-METHYLAMINO-L-ALANINE (BMAA) IS COMPLEX

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β -methylaminoalanine (BMAA) is a naturally occurring non-protein amino acid that was originally discovered in seeds of cycads (*Cycas marianensis*) that are traditionally eaten by the Chamorro people of Guam. The Chamorro experienced an epidemic of progressive neurodegenerative disease from the 1940s – present. It was hypothesized that BMAA may be biomagnified in foods and BMAA was found in autopsy brain samples of Chamorro patients who died of the disease as well as cyanobacteria symbionts of the cycads. Other researchers did not detect BMAA in cyanobacteria or human samples leading to controversy. We found that detection of BMAA by mass spectrometry as the predicted 119 m/z signal in electrospray ionization accounted for only 3% - 10% of the standard. The remaining >90% was found as a series of metal adducts, solvent interactions and a dimer. We synthesized Zn(BMAA)₂ as an alternate standard for determination of elution time and detected m/z. We evaluated a series of Spirulina products by 3 different BMAA analysis methods and found both positive and negative results for the identical samples. Together, these studies demonstrated that failure to detect BMAA in complex samples is not proof of absence of the compound.

C-4

SIX AROGENATE DEHYDRATASES SYNTHESIZE PHENYLALANINE IN *ARABIDOPSIS THALIANA*

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AROGENATE DEHYDRATASES (ADTs) catalyze the last step in the synthesis of the aromatic amino acid phenylalanine dehydrating/decarboxylating aroenate to phenylalanine. In *Arabidopsis thaliana* six independent genomic loci have been identified to code for *AtADTs*. All six enzymes share the same domain structure with an N-terminal transit peptide, an internal enzymatic domain and a C-terminal ACT or regulatory domain. With the exception of the transit peptide sequences, plant ADTs closely resemble PREPHENATE DEHYDRATASE (PDT) from bacteria and fungi. Although similar on the sequence level we have been able to identify differences in the biological roles of *Arabidopsis* ADTs. For example, *AtADTs* have unique enzymatic properties, their RNA expression patterns are tissue specific and they can respond to different environmental stresses. Furthermore we have transiently expressed *AtADTs* as CFP and YFP fusion proteins in *Arabidopsis* and *Nicotiana benthamiana* and it was determined that their

subcellular localization patterns are not identical and that they are able to interact with other proteins. This suggests that *AtADTs* are regulated differentially either at the transcriptional or post-translational level. In addition some evidence suggests that *AtADTs* might have non-enzymatic biological functions.

C-5

COLORIMETRIC BEHAVIOR OF XYLEM SAP OBTAINED BY MECHANICAL SQUEEZE FROM SILVER BIRCH (*BETULA PENDULA*) AND ITS COLOR CONTROL

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Sap is a fluid which plant utilizes to maintain its homeostasis and transportation of water and nutrients throughout the xylem. Fresh birch exudate (*Betula pendula*) is crystal clear liquid at room temperature for several days. We found that the discoloration occurred in fresh birch sap in hourly pace when obtained by mechanical squeeze. Interestingly this discoloration did not occur in the sap from other wood species (spruce, *Picea abies*). This study especially focused on the color development in the squeezed sap by mechanical compression. Enzymatic oxidation by polyphenol oxidase (PPO) was suspected as cause of the color development. N₂ gas and conventional enzyme inhibitors, Na₂SO₃ and ethylenediaminetetraacetic acid (EDTA) were applied. Results showed that Na₂SO₃ was the most effective inhibitor compared to the others. Chemical characterization in birch and spruce xylem sap in different seasons were also conducted. Knowledge on the differences between squeezed and exuded sap is important since squeezing can be envisaged as a pre-treatment step for any process – such as a biorefinery – that utilizes biomass and has the privilege of accessing trees that have recently been felled.

C-6

PROGRAMMING AND REPRORAMMING OF GENE EXPRESSION: MECHANISMS OF CELLULAR SPECIFIC METABOBLISMS?

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It has been estimated that plants in the plant kingdom can biosynthesize more than 200,000 plant secondary metabolites (PSM) (also, generally called plant natural products, PNP). Every single metabolite is synthesized via one main biosynthetic pathway maybe more than one branch. As understood in many biosynthetic pathways, PSM is in a manner of plant family, tissue, cell, development, and growth-specificity. The commonly known fact is that although genes required for the biosynthesis of a metabolite exist in the genome of plant, the biosynthetic pathway is only limited to certain specific cells. The mystery of this type of cellular specificity remains largely unknown. Numerous research efforts during the current post genome era have shown that biosynthetic pathways are generally controlled by the expression of transcription factors. However, many researches have also shown that the expression of transcription factor genes in transgenic plants may not lead to the formation of targeted metabolites even in the metabolic specific cells. Recently, our studies on anthocyanin biosynthesis have shown that the mechanisms underlying this type of observation are mostly likely associated with the requirement of a complete metabolic programming network activated in transgenic cells. We

isolated different types of metabolic specific cells from *Arabidopsis pap1-D* plants and *PAP1* transgenic tobacco plants, in which *PAP1* encoding a R2R3-MYB transcription factor is highly expressed to lead to the high production of anthocyanins. Cells isolated from *pap1-D* plants and *PAP1*-transgenic tobacco plants include red and white types. Although red and white cells are the same in the genetic context, red cells highly biosynthesize anthocyanins, while white cells do not produce anthocyanins or only produce trace levels of anthocyanins. To understand the mechanisms of the metabolic differentiations in these cells, genome-wide gene expression profiles are performed. The resulting data show the difference of transcriptional programming specifically required to anthocyanin biosynthesis in red and white cells. Red cells express *TTG1*, *GL3*, *TT8* and *PAP1*, the protein of which can form a WD40-bHLH-MYB (WBM) complex positively activating the biosynthetic pathway of anthocyanins, but white cells lack the expression of *TT8* and other genes. This result indicates that the biosynthesis of anthocyanins is the programmed consequence of the synchronized expression of multiple transcription factor genes. The genome-wide gene expression profiling analysis reveals that approximately 6.5% of genes in the *Arabidopsis* genome are altered in the expression patterns, many of which are uniquely expressed in red cells. We hypothesize that the alteration of the genome-wide gene expression results from a reprogramming consequence of the specific red cells and the resultant reprogramming are essential to maintain the anthocyanin biosynthesis. Although we have generally understood that plant specialized metabolisms are results of plant specific genes, our study indicates that specific metabolic programming and reprogramming control the final metabolic fate of cells.

C-7

NUDICAULINS, REVISED STRUCTURE AND BIOSYNTHESIS

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Papaver nudicaule (Iceland poppy) is an indigene of the northern landscape. Garden varieties occur with yellow, white, red or orange petals. The main pigments of the differently colored *P. nudicaule* varieties are nudicaulins (yellow), kaempferol (white), pelargonidin (red) or a mixture of those three (orange). The revised structure of the nudicaulin aglycon contains an indole part condensed with a polyphenolic-like moiety which bears a similar glycosidic conjugate pattern as the co-occurring kaempferol derivatives. The uncommon structure and unknown biosynthesis attracted the interest of our research. A retrobiosynthetic study with ¹³CO₂ demonstrated that the indole and polyphenolic part come from the indole/tryptophan pathway and the phenylpropanoid pathway, respectively. The biosynthesis was further investigated with feeding experiments of ¹³C-labelled precursors in suspensions of sliced petals. The results of feeding experiments with [U-¹³C]glucose showed that the *de novo* biosynthesis of nudicaulins takes place within the petals. This is in agreement with the ¹³CO₂ study. Further feeding experiments established the origin of the C₆-C₃-C₆ moiety from phenylalanine and acetic acid and the origin of the indole part from anthranilic acid and ribose. Analysis of various further yellow blooming poppy plants revealed nudicaulins as flower pigments of *Papaver alpinum* and *Meconopsis cambrica*.

C8

NEW ANTILEISHMANIAL AGENTS FROM PLANTS

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Bioassay guided fractionation of the methanol extract of *Terminalia arjuna* leaves led to the isolation of a new fatty alcohol with good antileishmanial activity along with ten known compounds: two phytosterols (Stigmasterol and β - Sitosterol 3-O-glucoside), five flavonoids (Apigenin , Luteolin , Vitexin , Isovitexin , Luteolin-3`-glucouronide), and three tannins (Gallic acid , Methyl Gallate and Ellagic acid). The new compound was identified to be (2E) 3, 5, 7, 16 tetramethyl heptadeca-2-en ol. The isolated fatty alcohol and its prepared acetate derivative showed antileishmanial activity with IC₅₀ values of 9.0 and 2.0 μ g/ml, respectively [Pentamidine control : IC₅₀ 2.1 μ g/mL]. Three new compounds had been isolated from *Cannabis sativa* L. and identified to be prenylated dihydrostilbene derivative (4,3'-dihydroxy, 5,4',5'-trimethoxy, 2-prenyl-dihydrostilbene), flavonoid Canniflavin C (5,7,4'-trihydroxy, 3'-methoxy,6-geranyl flavone), and 1,4 benzoquinone derivative (3-hydroxy, 5-pentyl,6-geranyl-1,4-benzoquinone). The isolated compounds exhibited very good antileishmanial activity (IC₅₀ values 3.5, 4.5 and 4.1 μ g/mL, respectively [Pentamidine control : IC₅₀ 2.1 μ g/mL]). Photooxygenation of Δ^8 -THC resulted in the isolation of several new compounds. One of them which is Δ^8 -THC quinone showed a powerful antileishmanial activity with IC₅₀ value of 0.06 μ g/mL.

Symposium II Genomics & Bioinformatics

S2-1

QUANTITATIVE GENOMICS OF NATURAL VARIATION IN PLANT METABOLISM: WHEN WILL THE COMPLEXITY END?

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Natural genetic variation and the resulting phenotypic variation between individuals within a species have been of longstanding interest in wide-ranging fields. In humans, natural genetic variation is frequently at the core of an individual's susceptibility to cancer and other debilitating disorders. In plants, natural genetic variation is the basis of plant breeding and an important foundation of ecology and evolution. The topic of natural genetic variation is also of critical importance to plants and their ability to respond to biotic stresses because a plants metabolic defense repertoire is highly variable across natural genotypes. This diversity provides both a complex impediment to designing the optimum genotype as well as a dramatic opportunity to utilize this diversity to address several questions. The first question of most direct interest is what are the genes underlying differences in how plants produce secondary defense metabolites? This same question and the answers obtained however also allows us to use this applied/ecological field of research to begin developing fundamental tools and quantitative genetic theory that can be applied to almost any phenotype. We have been using genomics methodologies to better understand how genetic diversity is controlled and identify the genes. In this presentation I will focus on how the combination of quantitative genetics and glucosinolate secondary metabolism is changing our understanding of the term "secondary" as well as how this novel metabolite class is illuminating what may be fundamental theories of quantitative genetics. In each case, the fundamental illumination as direct application consequences and the two cannot be separated. In the seminar I will focus on the following main areas: 1) Combining metabolomics and network expression analysis to identify the molecular basis of epistatic networks controlling plant secondary metabolism and how "secondary" is integrated into the whole plant; 2) A novel approach to identify causal candidate genes within genome wide association mapping at a greater than 75% success rate; and 3) Pathways are not really regulated as pathways.

S2-2

MAKING NEW MOLECULES

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Plants produce a huge array of natural products, many of which are specialised metabolites associated with particular species. These secondary metabolites often have important ecological roles, facilitating pollination and seed dispersal and/or providing protection against attack by pests and pathogens. Although the ability of plants to perform *in vivo* combinatorial chemistry by mixing, matching and evolving the genes required for different secondary metabolite biosynthetic pathways is likely to have been critical for survival and diversification of the Plant Kingdom we know very little about the mechanisms underpinning this process. This talk will focus on plant natural product function and synthesis, the origins of metabolic diversity and potential for metabolic engineering, drawing on our research on triterpene synthesis in crop and model plants.

S2-3

FATTY ACID COMPOSITION OF DEVELOPING SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) BERRY AND THE TRANSCRIPTOME OF THE MATURE SEED

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Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, fruit-producing plant known historically for its medicinal and nutraceutical properties. The most recognized product of sea buckthorn is its seed oil that is rich in essential fatty acids. Sea buckthorn is fast gaining popularity as a source of functional food but currently has few genomic resources. Therefore, we explored the fatty acid composition of Canadian-grown cultivars and the seed transcriptome using 454 sequencing. GC-MS profiling of fatty acids in seeds and pulp of berries indicated that the seed oil contained linoleic and α -linolenic acids at 33-36% and 30-36%, respectively, while the pulp oil contained palmitoleic acid at 32-42%. 454 sequencing of the cDNA from mature seeds yielded 500,392 sequence reads, which identified 89,141 putative unigenes represented by 37,482 contigs and 51,659 singletons. Gene Ontology and computational prediction of metabolic pathways indicated that primary metabolism and fatty acid biosynthesis pathways were highly represented categories. Sequences related to fatty acid biosynthesis genes in Arabidopsis were identified, and a subset of these was examined for transcript expression at four developing stages of the berry. This study provides the first comprehensive genomic resources represented by expressed sequences for sea buckthorn.

S2-4

PROFILING AND STRUCTURE ELUCIDATION OF SESQUITERPENE GLYCOSIDE ISOMERS FROM WILD TOMATO SPECIES *SOLANUM HABROCHAITES*

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Glandular trichomes are epidermal plant structures that are prolific chemical “factories” and accumulate substantial levels of specialized metabolites. Wild tomato *Solanum habrochaites* LA1777 and other accessions of the species possess a diverse set of storage/secretory glandular trichomes (SGTs) on the aerial surfaces of their leaves and stems that produce a diverse collection of chemicals, including numerous terpene isomers. This chemical diversity reflects a wealth of genetic resources that can be exploited to produce useful molecules. Recently, nonvolatile sesquiterpene glycosides were purified from LA1777 leaves, and their structures were elucidated using NMR and mass spectrometry. However, metabolite profiling indicated the presence of at least 10 isomers of some of these molecules across 15 different *S. habrochaites* accessions. Much of this isomerism is attributed to variation in sugar linkage chemistry, and was explored using both negative and positive ion mode mass spectrometry and collision induced dissociation. Three glycosylation patterns were identified among the molecules; glycosylation at

primary alcohol, secondary alcohol and by elongation of sugar chains. These results suggest functions for multiple glycosyltransferase enzymes in the conversion of sesquiterpenoids to nonvolatile forms.

S2-5

IDENTIFICATION AND CHARACTERIZATION OF GmMYB176 INTERACTOME IN SOYBEAN

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MYB transcription factors are one of the largest transcription factor families characterized in plants. They are classified into four types: R1 MYB, R2R3 MYB, R3 MYB and R4 MYB. GmMYB176 is an R1 MYB transcription factor that regulates *chalcone synthase (CHS8)* gene expression and isoflavonoid biosynthesis in soybean. Silencing of *GmMYB176* suppressed the expression of *CHS8* gene and reduced the accumulation of isoflavonoids in soybean hairy roots while the overexpression of *GmMYB176* did not alter both the target gene expression and metabolite level suggesting that GmMYB176 alone is not sufficient for *CHS8* gene regulation and it may act cooperatively with other factor (s) for *CHS8* gene activation in soybean. The current research is designed to identify and characterize the GmMYB176 interactome for *CHS8* gene regulation. GmMYB176 interacting proteins have been purified by over-expressing GmMYB176–YFP fusion proteins in soybean hairy roots, followed by co-immunoprecipitation. The purified GmMYB176 interacting proteins will be identified by LC-MS/MS analysis. Concurrently, yeast one hybrid assay is being used to identify GmMYB176 interacting proteins that possess direct DNA-protein interaction with the *CHS8* promoter. The findings of this research will provide a clearer idea about the proteins involved in regulation of *CHS8* gene expression and isoflavonoid biosynthesis.

S2-6

FUNCTIONAL GENOMIC INVESTIGATION OF EPHEDRINE ALKALOID BIOSYNTHESIS IN *EPHEDRA SINICA* – CLONING AND CHARACTERIZATION OF AMINOTRANSFERASES

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Ephedra sinica Stapf (Ephedraceae) is a perennial, broom-like shrub native to arid regions of China, Korea and Japan. This medicinal plant accumulates large amounts of the ephedrine alkaloids and has been utilized by humans for over 5,000 years. The ephedrine alkaloids, including (*S*)-cathinone, (1*R*,2*S*)-norephedrine, (1*R*,2*S*)-ephedrine and (1*S*,2*S*)-pseudoephedrine are analogs of amphetamine. When consumed by mammals these substances stimulate the

sympathetic nervous system, increasing blood glucose levels as well as heart and respiratory rates. While much is known about the pharmacological properties of these substances, the biological mechanisms by which they are synthesized remains largely unknown. A functional genomics platform was established in order to investigate biosynthesis of the pharmacoactive ephedrine alkaloids. RNA was extracted from young *Ephedra sinica* stems and sequenced by Illumina HiSeq2000 next-generation sequencing at The McGill University and Génome Québec Innovation Centre. Candidate biosynthetic enzymes were obtained from this EST collection based on homology to characterized enzymes with similar functions. This portion of the study is focused on identifying the Cathinone Aminotransferase/Synthase enzyme involved in the conversion of 1-phenyl-1,2-propanedione to (*S*)-cathinone. Twenty (20) candidates have been cloned and expressed in *E. coli* and are currently being assayed in order to functionally characterize enzymatic activities.

S2-7

COMPARATIVE BIOINFORMATICS OF A HIGH AJMALICINE LINE REVEALS COMPLEX REGULATION OF MONOTERPENOID INDOLE ALKALOID BIOSYNTHESIS IN CATHARANTHUS ROSEUS (L.) G. DON

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The Madagascar periwinkle [*Catharanthus roseus* (L.) G. Don] is a commercially important horticultural flower species and is the only source for several pharmaceutically valuable monoterpene indole alkaloids (MIAs), including the powerful antihypertensive ajmalicine and the antineoplastic agents vincristine and vinblastine. Screening of 3600 EMS mutagenized *C. roseus* plants for altered MIA profiles yielded one plant with high Ajmalicine content. Large scale sequencing and comparative bioinformatics of mutant and wild type plants showed up-regulation of the transcription factor ORCA3 and the enzyme Strictosidine beta-glucosidase (SGD) in the mutant line. Further biochemical and transcriptional characterization of this line suggests a mutation of an unknown regulatory element upstream of ORCA3, leading to the up-regulation of ORCA3 and SGD in the youngest leaves. The increased SGD activity in mutants increased the yohimbine MIA levels but not aspidosperma and iboga MIAs compared to the parental line. The study establishes that deep sequencing and comparative bioinformatics, in combination with molecular and biochemical characterization are valuable tools determining the genetic basis for mutations and events that trigger the MIA phenotype of this mutant. The value for breeding high MIA producing lines will be discussed.

Symposium III Bioproducts From the Canadian Forests, Production of Valued Attributes

S3-1

BIOPRODUCTS R&D WITHIN CANADA'S FOREST SECTOR INNOVATION SYSTEM: PLAYING A ROLE IN FOREST SECTOR TRANSFORMATION

Tom Rosser

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Over the past ten years the Government of Canada has worked with provinces, industry, academia and others to foster a strong and agile forest innovation system across the sector's value chain. Today's R&D capacity is aligned to enable Canada's forest sector to seize opportunities in the bioeconomy. New technologies are transforming the pulp and paper and solid wood industries, and being demonstrated, piloted or deployed at commercial scales. These include product innovations such as nanocrystalline cellulose produced at a pulp and paper plant in Quebec, and purified bio-methanol integrated with a kraft pulp mill in Alberta. Through FPInnovations, Canada's forest sector public-private partnership, the Government of Canada brings R&D capacity and targeted investments to innovation along the forest value chain, from seed to markets. Within this innovation framework, the Canadian Forest Service (CFS) of Natural Resources Canada (NRCan) is undertaking research in bioproducts to help discover new uses for forest-source chemicals in products currently dependent upon petrochemical sources. This CFS research is tightly linked with academic expertise at Western University and the University of Toronto, and with NRCan and FPInnovations research into biorefining. Additional support is provided by the NSERC Bioconversion Network and the Ontario Ministry of Economic Development and Innovation. With such forward-looking science that keeps watch for commercial application, Canada is well positioned to compete globally in the bioeconomy.

S3-2

MEDICINAL PLANTS AND PHYTOCHEMICALS FROM CANADIAN FORESTS

John T. Arnason¹, Jose A Guerrero¹, Andrew Waye¹, Jonathan Ferrier¹, Fida Ahmed¹, Carolina Cieniak¹, Ammar Saleem¹, Brendan Walshe-Roussel¹, Nan Shang², Steffany Bennett¹, Asim Muhammad¹, Vance Trudeau¹, Alain Cuerrier², Pierre Haddad²

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Recent work on medicinal plants from boreal and subarctic regions used by Cree and Inuit healers has led to the isolation of novel antidiabetic and neurologically active phytochemicals.

Altogether, five boreal species extracts were found to be active in animal models of diabetes and were investigated for active principles. From the bark of mountain ash, *Sorbus decora*, three new pentacyclic triterpenes were isolated and 23,28-dihydroxy-lupan-20(29)-en-3 β -caffeate was highly active in stimulation of glucose uptake in model systems. Tamarack, *Larix laricina* produced a novel triterpene, now named awashishic acid that stimulated adipogenesis in cell culture. Neurologically active phytochemicals were found in the pitcher plant, *Saracenia purpurea*, northern Labrador tea, *Rhododendron tormentosum* from Nunavut and a population of *Rhodiola rosea* from Ungava bay. In addition a survey of boreal and mixed deciduous tree extracts for activity in neurological receptor binding and enzyme assays shows that the potential for new phytochemical discoveries from these is high.

S3-3

PYROLYTIC CONVERSION OF BIOMASS RESIDUES INTO BIO-OIL AND BIO-CHAR: TURNING WASTE INTO BLACK GOLD

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The transformation of biomass into fuels and chemicals is becoming increasingly popular as a way to mitigate global warming and diversify energy sources. Biomass is a renewable, carbon-neutral resource which can be a source of valuable fuels, chemicals, pharmaceuticals and food additives through a variety of biochemical and thermochemical processes. The presentation will provide an overview of a number of projects that are being carried out at the *Institute for Chemicals and Fuels from Alternative Resources* (ICFAR), focusing particularly on thermal cracking technologies for the production of bio-oils and bio-char from a variety of agricultural, forestry and food residues and wastes, and on their possible utilization and upgrading routes. The presentation will place a special emphasis on the most innovative technologies that we have developed at ICFAR, including pilot plants based on (a) bubbling fluidized bed and (b) mechanically fluidized bed technologies. The presentation will also illustrate the progress towards the commercialization of pyrolysis processes for bio-oil and bio-char production.

S3-4

ENZYME TECHNOLOGIES FOR THE PRODUCTION OF HIGH-VALUE POLYMERS AND CHEMICALS FROM FOREST RESOURCES.

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The production of high value co-products can significantly improve the process economics of lignocellulosic biorefineries whose main product is bioenergy. This is particularly true for forest biorefineries located in northern countries like Canada that rely on slower growing trees that produce high-quality fibre. The aim of our research is to engineer biocatalysts (enzymes) that can be used to synthesize new, high-value polymers and chemicals from renewable plant materials. Examples of the technical bioproducts that we are working to develop include hemicellulose-derived coatings for food preservation, as well as biopesticides and nutraceuticals from wood-derived phytochemicals. Our enzyme approach to developing new, valuable polymers and chemicals from forest resources harnesses 1) the regio- and stereo-specificity of enzyme catalyzed reactions that have evolved to transform lignocellulosic substrates, and 2) our ability to manipulate protein function using genetic tools, to enhance or alter protein function. The catalytic specificity of enzymes is key to enhancing the bioactivity of wood biochemicals and tailoring the composition and performance of wood polysaccharides. Moreover, the aqueous and mild reaction requirements of enzyme catalyzed reactions can help retain the degree of polymerization of xylans and glucomannans recovered from pretreatment hydrolysates.

S3-5

**CONIFER SPECIALIZED DITERPENES: FROM CHEMICAL DEFENSE TO
PERFUME BIOPRODUCTS**

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Conifer trees produce large volumes of terpenoid oleoresin defenses for their protection against insects (e.g. bark beetles and weevils) and insect-associated fungal pathogens (e.g., ophiostomaoid fungi). Integrating genomics, transcriptomics, proteomics, and biochemical approaches, we have functionally characterized large gene families of terpenoid synthases (TPS-d family) and cytochrome P450-dependent monooxygenases (CYP720B family) of conifer oleoresin biosynthesis. It is the catalytic plasticity of these enzyme classes that drives the diversity of specialized metabolism in conifer defense, critical for the evolutionary adaptation of long-lived conifer trees in the light of changing environmental conditions. Beyond their immediate relevance for forest health, the functional landscape of conifer diterpene biosynthetic genes can be deployed for the improved production of various bioproducts with human benefit. Using metabolic pathway engineering in yeast and plant host systems, we are aiming to develop reliable production platforms for high-value diterpenes, such as diterpene resin acids and *cis*-abienol with applications in the resin and coatings or fragrance industry.

Symposium IV Botanicals & Medicinals

S4-1

CRANBERRY: A MODEL SYSTEM FOR UNDERSTANDING PHYTOCHEMICAL COMPLEXITY

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There is a long history of use and modern commercial importance of large (*Vaccinium macrocarpon* Aiton) and small cranberries (*V. oxycoccus* L. and *V. vitis-idaea* L) in North America. While epidemiological research indicates cranberries have positive health benefits, identifying specific phytochemicals for disease prevention remains elusive. The central objective of this research was to develop phytochemical characterization tools for comparing commercially cultivated cranberries and 2 wild-harvested *Vaccinium* species. A high performance liquid chromatographic method was developed and validated by AOAC International Guidelines to quantify cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, peonidin-3-O-galactoside and peonidin-3-O-arabinoside in cranberry fruit products. Previously undiscovered phytochemical complexity in cranberry was investigated by untargeted metabolomics by ultra-fast liquid chromatography-time of flight mass spectrometry. Multivariate data analysis, principal component and partial least squares discriminate analysis, with an application of univariate statistics to mitigate false discoveries demonstrated 8000 - 1000 phytochemicals and identified between 91 and 165 important new compounds in the species. Together, these data establish targeted and untargeted methods for phytochemical characterization of cranberries, providing foundational chemotaxonomic knowledge and new insights into the maintenance of health in traditional North American diets.

S4-2

PHYTOCHEMICALS AND METABOLIC SYNDROME

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Pharmacologically active, plant-derived polyphenols have been widely used in traditional medicine to treat and prevent diabetes. For example, an extract of Russian tarragon (*Artemisia dracunculus*), used as an anti-diabetic remedy in Eastern Europe, reduced hyperglycemia in animal models for type 2 diabetes and improved insulin sensitivity in treated clinical subjects. We have isolated active anti-diabetic polyphenols from this extract and putatively identified their molecular mode of action in the insulin-signaling pathway. In addition, we established that both genetic and epigenetic factors play decisive roles in the relative amounts of anti-diabetic compounds in *A. dracunculus*. Anthocyanin-rich berries have been also used as a traditional anti-diabetic medicine. Oral administration of blueberry and maqui berry (*Aristotelia chilensis*) anthocyanin-enriched extracts improved fasting blood glucose levels and glucose tolerance in hyperglycemic obese C57BL/6J mice fed a high fat diet. These data corroborate a recent clinical study conducted in patients treated with blueberry puree and suggest that blueberries may be a valuable dietary source of natural anti-diabetic compounds. The significant challenge facing food and supplement industry, however, is to deliver pharmacologically active doses of these compounds, without associated sugars, fiber and water. By leveraging the natural affinity of polyphenols for proteins, we have developed a method of using protein-rich food-based matrices, such as soy protein isolate, to efficiently sorb anthocyanins and other bioactive polyphenols from

juiced or extracted plant materials and showed that electrostatic binding to soy proteins enhances stability, bioavailability and efficacy of anti-diabetic polyphenols.

S4-3

GENE ANALYSIS OF 4' ACETOXY RESVERATROL: POTENTIAL HUMAN SKIN APPLICATIONS

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Resveratrol is naturally produced in abundance by several plants. As an effective natural compound in commercial products resveratrol has been problematic due to its rapid metabolism. To address this issue, structural modification has been performed on resveratrol to obtain a 4' acetoxy analog with enhanced physical/chemical properties. The purpose of this study: to investigate 4' acetoxy resveratrol (4AR) on the expression of skin genes and proteins using human dermal models and to determine whether 4AR may benefit skin health. 4AR at 1 % in qPCR experiments using a human skin model significantly increased gene expression of: 1) the anti-aging factor, SIRT 1 by 3.3-fold, 2) the extracellular matrix proteins- collagens (type III and IV), elastin and tissue inhibitors of metalloproteinases (TIMP 1 & 2), 3) anti-oxidants such as superoxide dismutase, LOX, and metallothioneins, and 4) growth factors- TGF beta, IGF-1, nerve growth factor, fibrillin, laminin and proliferating cell nuclear antigen. AR significantly down-regulated gene expression of inflammatory and skin-aging molecules, such as: IL-6, IL-8, COX-2, and the S100 calcium binding proteins A8 and A9. These findings suggest that 4AR has the potential to be used topically for the treatment and prevention of skin aging, by dramatically enhancing important dermal components.

S4-4

FLAVONOID COMPOSITION AND EXPRESSION ANALYSIS OF FLAVONOID BIOSYNTHESIS GENES IN THE ANTIOXIDANT RICH SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) BERRIES AND LEAVES

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Sea buckthorn (*Hippophae rhamnoides* L.) is fast gaining popularity as a source of nutraceutical food due to its high antioxidant properties and unique oil composition. Flavonoids are a class of plant phenolics with several beneficial effects on human health, including antioxidative effects. Phenolic compounds and flavonoids were identified by HPLC-UV in the leaves and developing berries (green, G; green/yellow, G/Y; yellow/orange, Y/O and orange/red, O/R) of four Canadian grown cultivars. The O/R stage berries of the RC-4 cultivar contained quercetin and myricetin levels (17.5 and 17.2 mg/100g FW) at nearly double the levels found in other cultivars, but the leaves contained remarkably higher levels of gallic acid, rutin, kaempferol (61-147 mg/100g FW), quercetin (63-105 mg/100g FW) and isorhamnetin (86 mg/100g FW) as compared to berries. We identified 406 unigenes encoding putative flavonoid biosynthesis enzymes in the transcriptome of sea buckthorn seed. RT-PCR analysis of 15 such genes at the four developmental stages of berries, as well as in the leaf tissue, showed that the expression of *C4H*, *CHI*, *UGT*, *OMT*, *LAR* and *ANR* was down regulated, while the expression of *4CL* and *F3'5'H* was the highest in the O/R stage berries. The significance of these results will be discussed.

S4-5

BLUEBERRY POLYPHENOL-ENRICHED SOYBEAN FLOUR REDUCES HYPERGLYCEMIA, BODY WEIGHT GAIN AND SERUM CHOLESTEROL IN MICE

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Defatted soybean flour (DSF) can sorb and concentrate anti-diabetic blueberry anthocyanins and other polyphenols, but not sugars. In this study blueberry polyphenol-enriched DSF (BB-DSF) or DSF alone were incorporated into the very high fat diet (VHFD) of obese, hyperglycemic C57BL/6 mice for 12 weeks to investigate anti-diabetic effects. Compared to the VHFD containing DSF, the diet supplemented with BB-DSF significantly reduced weight gain by 5.6%, improved glucose tolerance and lowered fasting blood glucose levels in mice within 7 weeks of intervention. Serum cholesterol was significantly lowered by 13.2% in mice consuming the diet supplemented with BB-DSF. Compounds were eluted from DSF and BB-DSF for *in vitro* mode of action experiments. Compared to untreated control, doses of BB-DSF eluate containing 0.05 – 10 µg/µL of blueberry anthocyanins significantly repressed glucose production by 24 - 74 % and improved insulin sensitivity in H4IIE rat hepatocytes. The results suggest that BB-DSF may be useful for the dietary management of pre-diabetes and/or diabetes, since it can deliver concentrated anti-diabetic fruit polyphenols without associated sugars and improve glucose metabolism.

S4-6

METABOLOMICS REVEALS EFFECTS OF XANTHOHUMOL ON MARKERS OF METABOLIC SYNDROME IN ZUCKER FATTY RATS

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The objective of this study was to determine the effects of xanthohumol (XN), a prenylated flavonoid from hops (*Humulus lupulus*), on markers of metabolic syndrome utilizing targeted and untargeted metabolomics. A 6 week treatment study was conducted in obese Zucker fa/fa rats. Animals were divided into four groups (n=12/group) and received the following doses of XN: 0, 1.86, 5.69, and 16.9 mg/kg body weight daily (equivalent to 0, 20, 60 and 180 mg in humans). A commercial mass spectrometry assay kit (Biocrates MetaDisIDQ™) was used to quantify 181 metabolites. Untargeted metabolomics was performed on an AB SCIEX 5600 Q-TOF instrument with information-dependent collection of MS/MS data. Both the targeted and untargeted metabolomics analyses revealed concerted and significant differences between XN treated and control groups. XN treatment had a dose-dependent lowering effect on plasma acylcarnitines, suggesting that XN promotes the alleviation of mitochondrial dysfunction by decreasing products of incomplete fatty acid oxidation. Untargeted metabolomics revealed treatment-related decreases in plasma allantoin, free fatty acid, hydroxyl fatty acid and dicarboxy fatty acid concentrations, all of which are elevated in type II diabetes. Taken together, the results suggest that XN has therapeutic potential in the treatment of metabolic syndrome and type II diabetes.

S4-7

INHIBITORY ACTIVITY OF SYNTHETIC SYRINGYL & GUAIACYL 8-O-4' NEOLIGNANS ON HUMAN CANCER CELL PROLIFERATION

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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, little biological activity of 8-O-4' neolignans on human health were studied, except for that 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 cancer prevent 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.

S4-8

PHYTOCHEMICAL SHIKONIN CAN INDUCE IMMUNOGENIC TUMOR CELL DEATH AND ENHANCE EFFICACY OF DENDRITIC CELL-BASED CANCER VACCINE

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Immunogenic cell death is characterized by damage-associated molecular patterns, which can enhance the maturation and antigen uptake of dendritic cells. Shikonin, an anti-inflammatory and anti-tumor phytochemical, was exploited here as an adjuvant for dendritic cell-based cancer vaccines via induction of immunogenic cell death. Shikonin can effectively activate both receptor- and mitochondria-mediated apoptosis and increase the expression of all five tested damage-associated molecular patterns in the resultant tumor cell lysates. The combination treatment with damage-associated molecular patterns and LPS activates test dendritic cells to a high maturation status and enhances the priming of Th1/Th17 effector cells. Shikonin-tumor cell lysate-loaded mature dendritic cells exhibit high level expression of CD86 and MHC class II and activate Th1 cells. The shikonin-tumor cell lysate-loaded dendritic cell vaccines result in a strong induction of cytotoxic activity of splenocytes against target tumor cells, a retardation in tumor growth, and an increase in the survival of test mice. The much enhanced immunogenicity and efficacy of the current cancer vaccine formulation, i.e, the use of shikonin-treated tumor cells as

cell lysates for pulse of dendritic cells in culture, may warrant future evaluation as an *ex vivo* clinical approach for developing individualized, dendritic cells-based anti-cancer vaccines.

S4-9

BIOLOGICAL ACTIVITY OF LECTINS DERIVED FROM MEDICINAL PLANTS

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The purpose of this report is to review our studies with plant lectins, a specific class of carbohydrate-binding proteins, focusing on lectins from medicinal plants. These lectins may serve as a supplementary or even key component of herbal medication influencing functions of many cells in the body and carrying their putative therapeutic activity. To address this issue, several tests with isolated human neutrophils were utilized to screen for biological activity of some rare lectins from medicinal plants (*Artocarpus heterophyllus*, *Caragana arborescens*, *Leucojum vernum*, *Sambucus nigra*, *Urtica dioica*, *Vicia sativa* and *Viscum album*) in comparison with well-known plant seed lectins from *Arachis hypogaea*, *Canavalia ensiformis*, *Phaseolus vulgaris*, and *Triticum vulgare*. The tests included measurements of cell aggregation and disaggregation (a measure of functionally active intercellular contacts), H₂O₂ generation (a measure of phagocyte NADPH-oxidase activity), and degranulation response (release of lysozyme). Results showed that lectins differentially stimulated the activity of neutrophils, revealing their potential to modulate innate immunity. In addition, several plant lectins stimulated the release of the lymphangiogenic factor VEGF-C from breast cancer cells. These findings strongly suggest that the presence and activity of lectins might be tested in plant medicinals and provide new approach for their assessment and standardization.

Symposium V Phytochemicals in the interaction between plants and their environment

S5-1

BELOWGROUND TERPENE METABOLISM: ORGANIZATION AND MULTIPLE FUNCTIONS

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Understanding the complexity of root phytochemistry is important to decipher belowground plant-organism interactions. We investigate the genomic, cell-specific, and subcellular organization of terpene specialized metabolism and its defensive functions in Arabidopsis roots. Roots of different Arabidopsis ecotypes produce volatile sesquiterpenes and a surprisingly diverse group of diterpenes. We show that the biosynthesis of these compounds is largely cell type-specific and exhibits some unexpected subcellular compartmentation. For example, roots of the Col ecotype produce unusual diterpene olefins named rhizathalenes, which are biosynthesized by the terpene synthase TPS08 in the root vascular tissue. Radial diffusion of rhizathalenes from the root stele mitigates herbivory on the surrounding cell layers. While we found diterpenes to be produced in root leucoplasts, we demonstrate that two root-specific sesquiterpene synthases are targeted to mitochondria indicating that these organelles represent an additional subcellular compartment in terpene specialized metabolism in Arabidopsis roots. Besides the biosynthesis of constitutively produced volatile terpenes, we investigate the root-specific, pathogen-induced formation of the common volatile homo(nor)terpenes. We have found that the homoterpene DMNT, in contrast to its formation aboveground, is derived from a triterpene biosynthetic route with suggested function in belowground defense.

S5-2

SQUALENE SYNTHASE AND THE ACCUMULATION OF STEROIDAL GLYCOALKALOIDS IN POTATO

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Squalene synthase (EC 2.5.1.2.1; SQS) catalyzes the head-to-head condensation of two molecules of farnesyl diphosphate to form the 30-carbon structure squalene. In all plants, SQS activity contributes to the formation of phytosterols, brassinosteroids, cholesterol, and steroidal glycoalkaloids (SGAs), which are secondary metabolites found most abundantly in plants of the genus *Solanum*. In the wild potato species *Solanum chacoense*, the abundance and structural profile of SGAs varies between the aerial, subterranean, and stoloniferous tissues. The SQS enzyme functions at a branch point in isoprenoid metabolism competing with sesquiterpene synthases for the soluble substrate to produce the hydrophobic hydrocarbon product. Unlike other eukaryotes, higher plants have more than one gene coding for SQS. *S. chacoense* accumulates transcript for at least three genes encoding SQS homologs. The tissue-specific pattern of transcript accumulation differs for each gene. The predicted polypeptides have 74 to 83% identity. Current research focuses on characterizing the activity of enzymes produced by heterologous expression in *Escherichia coli*. Each of the three genes has an intron in the 3'-

UTR. We are generating gene constructs with altered structures including the absence of introns and the introduction of premature stop codons to test the role of the intron in gene expression.

S5-3

ANTIFUNGAL METABOLITES FROM FUNGAL ENDOPHYTES OF *PINUS STROBUS*

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A collection of 86 fungal endophytes were isolated from the needles of *Pinus strobus* (eastern white pine) trees located in Eastern North America. DNA sequencing determined that the majority of the endophytes present in needles were species of *Lophodermium*. All isolated endophytes were grown in one litre cultures, extracted and tested for antifungal activity in disc diffusion assays. The five strains that showed the most activity were selected for further study with the goal of identifying antifungal compounds that might be active against white pine blister rust - the highly destructive introduced pathogen of pine trees. From these five isolates, a new aliphatic polyketide and three new biosynthetically related sesquiterpenes were isolated and characterized. Additionally, pyrenophorol, dihydropyrenophorin, and pyrenophorin (previously described macrolides) were isolated and identified. Their structures were elucidated by spectroscopic analyses including 2D NMR, HRMS and by comparison to literature data where available. Three of the isolated compounds were antifungal against both the rust *Microbotryum violaceum* and *Saccharomyces cerevisiae*, and pyrenophorol significantly reduced the growth of white pine blister rust in liquid culture.

S5-4

DEREPLICATION OF KNOWN PREGNANE GLYCOSIDES AND STRUCTURAL CHARACTERIZATION OF NOVEL PREGNANES IN *MARSDENIA TENACISSIMA* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND ELECTROSPRAY IONIZATION-TANDEM MASS SPECTROMETRY

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In the search for novel natural products in plants it is important to efficiently distinguish novel compounds from previously identified compounds, a process known as dereplication. In this study electrospray ionization-multiple stage tandem mass spectrometry (ESI-MSⁿ) was used to study the behaviour of twelve pregnane glycosides and genins previously isolated from *Marsdenia tenacissima*, a traditional Chinese medicinal plant, as a basis for dereplication of compounds in a plant extract. In addition to [M+Na]⁺ and [M+NH₄]⁺ ions, a characteristic [M-glycosyl+H]⁺ ion was observed in full-scan mode with in-source fragmentation. Sequential in-trap collision-induced dissociation (CID) of [M+Na]⁺ ions from 11,12-diesters revealed consistent preferred losses of substituents first from C-12, then from C-11. A crude methanol extract of *M. tenacissima* stems was analyzed by high performance liquid chromatography

(HPLC) coupled to ESI-MS. Several previously isolated pregnane glycosides were dereplicated and the presence of an additional nine novel pregnane glycosides is predicted based on the primary and fragment ions observed, including two with a previously unreported C₄H₇O C-11/C-12 substituent of pregnane glycosides. To our knowledge this study is the first report of prediction of the structures of novel pregnane glycosides in a crude plant extract by a combination of in-source fragmentation and in-trap CID.

S5-5

PHYTOCHEMICAL CONTENT OF WILD ATLANTIC CANADIAN LUPINS (*LUPINUS POLYPHYLLUS* L.): OPPORTUNITIES FOR BIOPRODUCTS AND CROP DIVERSIFICATION.

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While not native to eastern North America, wild lupin (*Lupinus polyphyllus* L.), is particularly well suited to the cool, wet maritime climate and acidic soils present in the region. In as much, showing an invasive growth habit and flourishing along roadsides, coastal stretches and other non-cultivated lands, thanks to a combination of resistance to local pathogens/herbivores and nitrogen fixation activity, efforts should be directed towards utilizing this plant instead of eradicating it. Phytochemical profile characterization of wild lupins via UPLC-MS and NMR methods, including flavonoids (flavones, isoflavones, anthocyanins), quinolizidine alkaloids, and seed oil contents has been conducted. Potential bioproducts and/or biofumigants opportunities, as a part of existing crop rotation systems in the region are being assessed.

S5-6

WAXES COATING THE SURFACES OF PETALS: ARE THEY DIFFERENT FROM THOSE ON VEGETATIVE ORGANS?

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Cuticles coating petals likely serve dual physiological and ecological roles by protecting the reproductive organ against environmental stress and mediating interactions with pollinators. Understanding such functions requires knowledge of the composition. We have carried out a first comprehensive investigation into the chemical composition and physiological properties of petals, using *Cosmos bipinnatus* (Asteraceae) ray flowers as a model system. In the wax mixture, four uncommon classes of very-long-chain compounds were identified and quantified. The first two classes were homologous series of alkane 1,2-diols and 1,3-diols, both ranging from C₂₀ to C₂₆, the other two were homologous series of primary and secondary monoacetates of C₂₀ to C₂₄ 1,2-diols. Besides, compound classes commonly found in leaf waxes were identified, including homologous series of alkanes, primary alcohols, fatty acids and esters, as well as triterpenoids. The relative quantities of these components differed between the adaxial and abaxial sides of the petal. The petal surface waxes had higher permeability for water than the average leaf permeabilities reported to date.

S5-7

PROGRESS TOWARDS RAPID IDENTIFICATION OF PHYTOCHEMICALS IN PLANT EXTRACTS

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New mass spectrometry equipment is bringing closer to reality the rapid accurate assessment of chemical composition of extracts from a variety of plant materials. Using a variety of plant sources, we are using HPLC separation, UV-VIS spectrometry, ion trap mass fragmentation and accurate mass determination to quickly “fingerprint” plant extracts. Extracts from citrus, eastern red cedar, and brassica species are being evaluated. *Camelina sativa* (L. Crantz) seedmeals have been evaluated for their glucosinolate, flavonoid, and phenolic phytochemical composition by a combination of MS and NMR methods. The goal is to use this information in collaboration with instrument software and internet databases to quickly determine phytochemical composition. Accurate phytochemical analysis can then be translated into rapid non-destructive analytical methods, such as near infrared spectrometry, for rapid evaluation of agricultural crops and processed fractions.

S5-8

NOVEL PYROLYSIS APPLICATIONS FOR BIOMASS CONVERSION AND NATURAL PRODUCT RECOVERY

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Thermochemical conversion of biomass yields bio-oils and valuable chemicals. Improvements to pyrolysis were sought to provide a cost-effective process to isolate and extract “known” plant natural products from agriculture or forestry residue. A mechanically fluidized reactor (MFR) was designed to: 1) separate selected compounds by pyrolysis temperature “cuts” and 2) increase the heating rate for isolating compounds from crop residue, including flavonoids, alkaloids and glucosinolates. Nicotine was identified in tobacco bio-oil from pyrolysis > 500 °C, but sinigrin was not detected after pyrolysis of mustard straw. Flavonoids survived pyrolysis temperatures < 200°C, but were not identified in the bio-oil. A comparison of lignin bio-oil cuts collected every 50°C from ambient up to 300°C with 5 and 15°C /min heating rates found that the faster rate produced the most bioactive cut at 250 and 300°C. These results highlight the importance of optimizing the MFR operating conditions in order to separate active compounds formed from the pyrolysis of lignin and potentially other interesting biomass. The MFR can be used to selectively prepare bio-oil cuts at relatively narrow temperature ranges (50°C), but the chemical characteristics of the selected compounds will influence whether they will survive pyrolysis temperatures.

Poster Abstracts

P-01

TWO HOMOLOGOUS RNA-BINDING PROTEINS PLAY DIFFERENT ROLES IN CAROTENOID BIOSYNTHESIS AND STRESS ADAPTATION IN *LOTUS JAPONICUS*

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In plants, RNA-binding proteins (RBPs) play critical roles in all aspects of post-transcriptional gene regulation, which affects various developmental processes and adaptation to environmental conditions. We identified two homologous RBPs, *Lj-RBP47Ca* and *Lj-RBP47Cb*, from *Lotus japonicus*. Two independent transgenic RNAi lines of *Lj-RBP47C* showed enhanced levels of carotenoids accompanied by increased expression of phytoene synthase and lycopene β -cyclase relative to the wild type control. We also found *Lj-RBP47Ca* to be regulated by light and gibberellic acid relative to *Lj-RBP47Cb*, which was induced more by various biotic and abiotic stresses. *Lj-RBP47C* promoter::GUS fusion experiments revealed that *Lj-RBP47Cb* had a more ubiquitous expression pattern compared to *Lj-RBP47Ca*. Taken together, our results indicate *RBP47Ca* may have a role in the biosynthesis of carotenoids by repressing *PSY* expression, whereas *Lj-RBP47Cb* may be involved in plant response to various abiotic and biotic stresses.

P-02

COMPARATIVE EVALUATION OF SOFTWARE FOR ANALYSIS OF GC-MS METABOLOMICS DATA

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The data analysis work-flow of an untargeted metabolomics experiment using GC-MS normally includes the following steps: deconvolution of chromatographic peaks, alignment of chromatographic peaks, peak picking and area integration, statistical analysis, and chemical identification of statistically significant chromatographic peaks. Several software packages have been developed in recent years to automate several or all of these steps. This work reports a comparison of the performance of ten programs: MetaboliteDetector, SpectConnect, MetAlign, MetIDEA, MeltDB, Metaboanalyst, Mzmine, TagFinder, XCMS, and GaVIN. In the first trial four groups of mixtures, with four replicates in each group, were prepared using 72 commercially available chemicals. In the second trial a non-polar extract of tomato vines was either spiked or left un-spiked with a mixture of alkanes. The samples were analysed by GC-MS, and the software packages, in combination with principal components analysis, were assessed for their ability to correctly identify peaks that differed between groups of samples. Of the ten programs tested, GaVIN, MetaboliteDetector, and MetAlign gave the best performance overall.

P-03

HETEROLOGOUS EXPRESSION OF GRAPEFRUIT CLONES PGT3 AND PGT9 IN YEAST SCREENING OF RECOMBINANT PROTEIN FOR ACTIVITY

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The wide diversity of plant secondary products results from different modifications undergone during biosynthesis, including glucosylation. These modification reactions result in production of the compounds actually found in plants and to unique chemical and biochemical properties, including some bitter compounds in grapefruit. While the presence of a PSPG box motif allows for identification of a clone as a putative glucosyltransferase (PGT), diversity of GT primary structures makes it difficult to accurately assign specific function. Our approach is to identify and isolate putative GT clones, express them heterologously, and biochemically characterize the proteins. Eleven putative GT clones have been isolated from *Citrus paradisi* and some have been biochemically characterized. The current hypothesis being tested is that PGT3 and PGT9 clones are plant secondary product GTs. Due to issues with inclusion bodies when using *E. coli*, proteins were expressed in *Pichia pastoris* using the pPICZA vector. Recombinant protein expression was confirmed by Western blot and proteins were enriched by IMAC. Over 30 flavonoid and simple phenolic substrates, representing many compounds found in grapefruit, were screened for activity with PGT3 and PGT9 proteins. No significant activity was found and the biochemical function of the proteins encoded by these clones will be further investigated.

P-04

SOYBEAN CYCLOPHILIN GENE FAMILY: CHARACTERIZATION OF *GmCYP1*

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Cyclophilins (CYPs) belong to the immunophilin superfamily with peptidyl-prolyl *cis-trans* isomerase (PPIase) activity. PPIase catalyzes the interconversion of the *cis*- and *trans*-rotamers of the peptidyl-prolyl amide bond of peptides. By *in silico* analysis, we identified 62 different cyclophilin genes in soybean (*GmCYP1* to *GmCYP62*) of which 8 are multidomain and 54 are single domain proteins. At least 50% of the *GmCYP* genes are expressed in soybean, as they have 99-100% sequence identity with soybean Expressed Sequence Tags. Out of 62 *GmCYPs*, *GmCYP1* interacts with *GmMYB176* both in yeast two hybrid assay and *in planta* where strong interaction was seen in the nucleus. Furthermore, *GmCYP1* localizes in the nucleus and the cytoplasm. Nuclear localization of *GmCYP1* might be associated with its interaction with *GmMYB176*, as the latter primarily localizes in the nucleus for regulating *CHS8* gene expression in soybean. We speculate that the interaction of *GmCYP1* with *GmMYB176* may possibly indicate a role of *GmCYP1* in isoflavonoid biosynthesis in soybean.

P-05

LIGNIN BIOSYNTHESIS THROUGH *IN VITRO* PEROXIDASE-CATALYZED DEHYDROGENATIVE POLYMERIZATION WITH CONTROLLING FEEDING RATIOS OF CONIFERYL AND SINAPYL ALCOHOL

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A dehydrogenative polymer (DHP) was synthesized *in vitro* through dehydrogenative polymerization using different feeding ratios of coniferyl alcohol (CA) and sinapyl alcohol (SA) (10:0, 8:2, 6:4, 2:8, 0:10) to understand monolignol coupling mechanism in the presence of horseradish peroxidase (HRP), *Coprinus cinereus* peroxidase (CiP) or soybean peroxidase (SBP) with H₂O₂. The turnover capacities of HRP, CiP and SBP were measured for coniferyl alcohol (CA) and sinapyl alcohol (SA), and CiP and SBP were found to have the highest turnover capacity for CA and SA, respectively. The yields of HRP-catalyzed DHP (DHP-H) and CiP-catalyzed DHP (DHP-C) were estimated between ca. 7% and 72% based on the original feeding weights of CA/SA in all synthetic conditions. However, a much lower yield of SBP-catalyzed DHP (DHP-S) was produced compared to that of DHP-H and DHP-C. In general, the DHP yields gradually increased as the feeding ratio of CA/SA increased. The average molecular weight of DHP-H also increased with increasing CA/SA ratios, while those of DHP-C and DHP-S were not influenced by the feeding ratios. The frequency of β -O-4 linkages in the DHPs decreased with increasing CA/SA ratios, indicating that formation of β -O-4 linkages during DHP synthesis could be influenced by peroxidase types.

P-06

IDENTIFICATION AND CHARACTERIZATION OF A *CYSTEINE PROTEASE* GENE IN TOBACCO FOR USE IN RECOMBINANT PROTEIN PRODUCTION

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A large number of human recombinant proteins of therapeutic value have been successfully produced in plant systems. The main technical challenge in the field of recombinant protein production is to produce sufficient level of proteins in plants. Proteolytic degradation of recombinant proteins has been a major problem both *in planta* during protein expression as well as *ex planta* during extraction and processing. One approach to overcome proteolytic degradation involves the creation of stable transgenic plant lines with reduced proteolytic activity. We have found that cysteine protease (CysP) inhibitors show protective effect on human immune-regulatory interleukin-10 (IL-10) production in plants. To identify the CysP involved in transgene accumulation in plants, we searched the DFCI tobacco expressed sequence tag database for *CysP* genes. This process identified a total of 32 tentative contig (TC) sequences and 23 singletons. An *in silico* expression analysis of the TC sequences identified 5 candidate *CysPs* based on tissue specific expression pattern. *CysP* gene silencing constructs were created to suppress the expression of individual *CysP* genes using RNAi silencing mechanism in transgenic tobacco line that overexpresses IL-10 protein. Agrobacteria-mediated plant transformation technology was utilized to generate transgenic lines with reduced CysP and increased IL-10 accumulation.

P-07

DIRECTED BIOSYNTHESIS OF ALKALOID ANALOGS IN *BERBERIS VULGARIS*

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Precursor directed biosynthesis (PDB) is the incorporation unnatural starting materials to yield novel natural products, often containing unusual functional groups. Potentially useful compounds can be generated through PDB with greater ease than afforded by total synthesis approaches. All benzyloisoquinoline alkaloids (BIAs), representing over 2500 compounds, many of which have potent pharmacological activity, are generated from the two precursors dopamine (DA) and 4-hydroxyphenylacetaldehyde (4HPAA). It was not clear whether BIA biosynthesis pathways in plants can utilize unnatural substrates. Thus, to explore the native potential of BIA pathways for PDB, we prepared a series of halogenated DA and 4HPAA analogs. These compounds were fed to callus cultures of *Berberis vulgaris*, a source of the antimicrobial BIA berberine. LC-MS analysis of the resulting cell extracts reveals production of some halogenated berberine analogs as well and limits of PDB this system.

P-08

BACTERIAL LACCASE EXPRESSED USING MICROAEROBIC CULTIVATION DISPLAYS OXIDATIVE ACTIVITY ON ANTIOXIDANT PHENOLIC COMPOUNDS

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Phenolic compounds with desirable antioxidant activities do not always have the desired bioavailability due to solubility constraints. One solution to this constraint may be found in laccase enzymes, which use a copper cofactor to catalyze oxidation of aromatic substrates to produce phenoxy radicals. These radicals can then undergo non-enzymatic reactions to produce coupled products. Therefore, this research aims to use laccases to form a phenolic with increased lipophilicity to enhance bioavailability. A bacterial laccase (SCO6712) from *S. coelicolor* is theoretically expected to have a Cu: (protein monomer) molar ratio of four. When producing SCO6712 in *E. coli* under microaerobic conditions rather than fully aerobic growth conditions, the ratio of moles copper to moles of protein increased from 0.8 to 1.19. Microaerobically produced point mutants of SCO6712, at active site residues involved in binding copper, did not always show increase in copper binding, when compared to their aerobically grown counterparts. Microaerobically produced SCO6712 showed activity on 11 of 24 tested phenolic compounds, including the phytochemical antioxidants resveratrol and the flavonols (kaempferol, quercetin and myricetin). Future experiments will attempt to isolate and characterize the oxidized products and analyze resultant changes in antioxidant activities of the phenolics.

P-09

RECOMBINATION AND SCREENING OF PUTATIVE GLUCOSYLTRANSFERASE 4 IN *PICHTIA PASTORIS*

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Flavonoids are a group of plant secondary metabolites that are vital to the cell systems of plants. The intake of these chemicals is advantageous to animals for their antioxidant properties that affect the function of immune and inflammatory cells. The bitter taste of grapefruit (*Citrus paradisi*) and other citrus species is caused by the accumulation of glycosylated flavonoids. Glucosyltransferases (GTs) are enzymes that add glucose moieties to a carbon or hydroxyl group of natural products. The function of a putative secondary product GT clone was tested. In previous research, putative GT 4 was cloned into a pCD1 modified pET expression system, heterologously expressed in *E. coli*, and screened for activity with only a few substrates, and little GT activity was found. Issues of protein localized to inclusion bodies in bacteria are being addressed. PGT 4 is being heterologously expressed in yeast (*Pichia pastoris*) to allow for protein production and analysis. PGT 4 will be screened for GT activity with different flavonoid subclass representatives and simple phenolics. PGT 4's significant impact on the biochemical regulation of *Citrus paradisi* will be elucidated with its characterization and determination of PGT 4's structure and function.

P-10

HAIRY ROOTS AS A MODEL TO INVESTIGATE THE ROLE OF SUBERIN IN THE *PHYTOPHTHORA SOJAE*-SOYBEAN PATHOSYSTEM

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Part of the innate resistance of soybean (*Glycine max* [L.] Merr.) to *P. sojae* involves pre-formed root suberin. Hairy roots, which form as a result of *Agrobacterium rhizogenes* infection/transformation, can be used to generate genetically modified adventitious roots with modified amounts of suberin and used to study the role of soybean root suberin in the *P. sojae*-soybean pathosystem. However, it is necessary to establish whether hairy root anatomy, suberin chemistry and response to pathogen challenge mimics that of wild type roots. To do this, hairy root cultures were established and their anatomy, and suberin deposition pattern examined using both fluorescent and non-fluorescent dyes targeting suberin. While the overall root anatomy and suberin deposition pattern of hairy roots was similar to that of wild type, the cortical cells were more loosely packed and displayed large intercellular spaces. The chemical composition and amount of suberin in soybean hairy roots, quantified using gas chromatography-mass spectrometry, was the same as wild-type soybean roots, with the amount of suberin increasing in both epidermal and endodermal cells along the root axis. Finally, the response of soybean hairy roots to *P. sojae* infection was investigated and also found to be similar to that of wild type roots.

P-11

CAPILLARY ELECTROPHORESIS TO QUANTITATE GOSSYPOL ENANTIOMERS IN COTTON FLOWER PETALS AND SEED

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Gossypol is a toxic compound that occurs as a mixture of enantiomers in cotton plant tissues including seed and flower petals. The (-)-enantiomer is more toxic to non-ruminant animals. Efforts to breed cottonseed with a low percentage of (-)-gossypol requires the determination of the (+)- to (-)-gossypol ratio in seed and flower petals. We have developed a method to quantitatively determine the total gossypol, and percent of its enantiomers in cotton tissues using high performance capillary electrophoresis (HPCE). The method utilizes a borate buffer at pH 9.3 using a capillary with internal diameter of 50 μ , effective length of 24.5 cm, 15 kV and cassette temperature of 15°C. This method provides high accuracy and reproducible results with a limit of detection of the individual enantiomers of less than 36 ng/mL, and provides base line separation of the enantiomers in a run time of <6 minutes.

P-12

FUNCTIONAL ANALYSIS OF ARABIDOPSIS WILL DIE SLOWLY GENES Kristina Sabou¹, Adel Al-Shammari¹, Xiaoquan Xiong¹, Shulan Sun², Xiaojing Wang², Owen Rowland¹, Tim Xing¹

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Programmed cell death (PCD) is a form of cellular suicide and involves a set of biochemical programs during the development of organisms and in their responses to pathogen attacks and other stress signals. We have isolated and characterized the *Will Die Slowly (WDS)* gene family of *Arabidopsis thaliana*. Molecular genetics and biochemical analysis has suggested a complex network involving some members of this gene family.

P-13

***TaFLRS*, A NOVEL MITOGEN-ACTIVATED PROTEIN KINASE IN WHEAT DEFENSE RESPONSES**

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Plants respond to biotic and abiotic stresses through the activation and coordination of various signaling pathways. The activation often requires the phosphorylation of proteins. Our results indicate that *TaFLRS* is transcriptionally upregulated in incompatible interactions involving wheat and leaf rust and *Fusarium graminearum*, suggesting that this MAPK maybe involved in defense responses to these wheat pathogens. RT-PCR revealed that *TaFLRS* transcript levels are not altered by salicylic acid (SA) treatment. However, immunoprecipitation and western blotting analysis show that phosphorylation of TaFLRS at the TEY motif was enhanced by SA in the Fusarium head blight (FHB) resistant cultivar Frontana following challenge with the FHB pathogen. The role of TaFLRS MAP kinase in defense responses in wheat is discussed.

P-14

DETERMINING SUBCELLULAR LOCALIZATION OF AROGENATE DEHYDRATASES USING CFP FUSION PROTEINS

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The aromatic amino acid phenylalanine (phe) can only be synthesized by plants and microorganisms while humans and other animals must rely on dietary intake. In plants aside from its role in protein synthesis, phe is used as a precursor for phenylpropanoids, a diverse family of organic compounds which are used for structural polymers, UV protection, stress responses, pigmentation or scent molecules. In plants the final step of phe biosynthesis is catalyzed by AROGENATE DEHYDRATASES (ADTs), enzymes that dehydrate and decarboxylate arogenate to phe. There are six ADTs encoded in the *Arabidopsis thaliana* genome, all sharing a common domain structure. All six *AtADTs* were cloned in frame with the coding sequence for the cyan fluorescent protein (CFP). They were introduced by Agrobacterium infiltration into the leaves of *Nicotiana benthamiana* and the transient expression of the ADT-CFP fusion proteins was monitored by confocal microscopy. Predominantly, ADT-CFP fusion proteins localized to projections from chloroplasts known as stromules. This is consistent with their enzymatic role as stromules are predicted to support the transport of molecules from the chloroplast to the cytosol facilitating the use of phe for protein synthesis. In addition we will discuss potential non-enzymatic roles of *AtADTs*.

P-15

TANSHINONES AS SELECTIVE AND SLOW-BINDING INHIBITORS FOR SARS-COV CYSTEINE PROTEASES

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In the search for SARS, tanshinones derived from *Salvia miltiorrhiza* were found to be specific and selective inhibitors for the SARS-CoV 3CL^{pro} and PL^{pro} viral cysteine proteases with deubiquitination activity. A literature search for studies involving the seven isolated tanshinone hits showed that at present, none have been identified as coronaviral protease inhibitors. We have identified that all of the isolated tanshinones are good inhibitors of both cysteine proteases. However, their activity was slightly affected by subtle changes in structure and targeting enzymes. All isolated compounds (**1–7**) act as time dependent inhibitors of PL^{pro}, but no improved inhibition was observed following preincubation with the 3CL^{pro}. In a detail kinetic mechanism study, all of the tanshinones except rosmariquinone (**7**) were identified as noncompetitive enzyme isomerization inhibitors. However, rosmariquinone (**7**) showed a different kinetic mechanism through mixed-type simple reversible slow-binding inhibition. Furthermore, tanshinone I (**5**) exhibited the most potent nanomolar level inhibitory activity toward deubiquitinating (IC₅₀ = 0.7 μ M). Additionally, the inhibition is selective because these compounds do not exert significant inhibitory effects against other proteases including chymotrysin, papain, and HIV protease. These findings illustrate the need to develop innovative inhibitors for SARS-CoV viral infection and replication.

P-16

EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF TWO GLUCOSYLTRANSFERASES FROM *CITRUS PARADISI*

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Glucosylation is a common alteration reaction in plant metabolism and is regularly associated with the production of secondary metabolites. Glucosylation serves a number of roles within metabolism including: stabilizing structures, affecting solubility, transport, and regulating the bioavailability of the compounds for other metabolic processes. The enzymes that lead to glucoside formation are known as glucosyltransferases (GTs), and characteristically accomplish this task by transferring a UDP-activated glucose to a corresponding acceptor molecule. GTs involved in secondary metabolism share a conserved 44 amino acid residue motif (60–80% identity) known as the plant secondary product glucosyltransferase (PSPG) box, which has been demonstrated to include the UDP-sugar binding moiety. Among the secondary metabolites, flavonoid glycosides and limonoid glycosides affect taste characteristics in citrus making the associated glucosyltransferases particularly interesting targets for biotechnology applications in these species. The research focus of our lab is to establish the function of putative secondary product glucosyltransferase clones identified from *Citrus paradisi*. In the present study, we report on the activity and biochemical characterization of two clones, PGT 7 (Flavonol-3-O-GT) and PGT8 (Limonoid GT) which were expressed in *Pichia pastoris*.

P-17

**OLEUROPEYL GLUCOSE ESTERS FROM THE SECRETORY CAVITIES OF
*EUCALYPTUS***

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The large and economically important genus *Eucalyptus* is well known for the abundant and diverse secondary metabolites its trees can produce. In particular, the mono- and sesquiterpenes stored in specialised secretory cavities embedded within leaves are characteristic of the genus. Recently, numerous oleuropeyl glucose esters have also been isolated from within the secretory cavities of eucalypts. To begin to understand the functional role of these esters within the cavities a systematic survey of the genus was undertaken to assess their structural diversity and prevalence within secretory cavities. We used the unique MS2 fragmentation pattern of the esters to identify numerous known and novel compounds from a wide range of species. The sequestration of oleuropeyl glucose esters to the extracellular domain of secretory cavities is suggestive of potential functional roles. Moreover, the fact that secretory cavities can be isolated from within eucalypt leaves is likely to aid future work on the biosynthesis of this interesting class of compounds.

P-18

**QUALITY CONTROL OF *CRATAEGUS PINNATIFIDA* BASED ON HIGH
PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTOR**

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Quality control of various herbal medicines is very important to ensure their safety and efficacy. *Crataegus pinnatifida* (Rosaceae) is widely used as a herbal medicine to improve digestion, remove retention of food, promote blood circulation and resolve blood stasis in Korea and China. In order to compare quality of *C. pinnatifida* of different region and origin, validated HPLC-DAD method was applied to analyze *C. pinnatifida* samples. We selected two compounds, chlorogenic acid and hyperin as marker compounds. We collected 30 samples of different cultivation region and origin. These samples were analyzed by HPLC method and contents of chlorogenic acid and hyperin in these samples were calculated, respectively. The content of chlorogenic ranged from 0.008 to 0.08 % and the hyperin content fell in the range 0.003 to 0.12%.

P-19

MONITORING FOR THE QUALITY EVALUATION OF *SINOMENIUM ACUTUM* PRODUCTS

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Sinomenium acutum (Menispermaceae) has been used as a traditional herbal medicine for the treatment of rheumatoid arthritis, arrhythmia, and pain and were collected from east and south Asia. Different cultivation, origins and harvest time affect the quality of herbal medicine. We evaluated the quality of *S. acutum* extract through developed simultaneous determination of sinomenine, magnoflorine and syringaresinol by HPLC-DAD. Quality evaluation successfully applied to quantify the three compounds in 30 samples from different localities in Korea and China. The identification of these compounds in *S. acutum* samples was based on the retention time and the comparison of UV spectra with standard compounds. The three compounds, sinomenine, magnoflorin and syringaresinol examined ranged from 0.005 to 15.795 mg/g.

P-20

MOLECULAR ANALYSIS OF A REPRESSOR-LIKE MYB FACTOR IN POPLAR

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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. Poplar trees (*Populus* spp.) accumulate PAs in vegetative organs including roots and leaves, and in some poplar species, PA biosynthesis genes are activated following herbivore damage, mechanical wounding, and light stress. Recently, the stress-inducible transcription factor MYB134 was isolated and shown to regulate PA synthesis in poplar. Constitutive expression of MYB134 in transgenic poplar resulted in the specific activation of PA pathway genes, leading to a dramatic increase in PA concentration. Furthermore, microarray analysis of the transgenic plants showed that not only structural genes, but also previously uncharacterized regulatory genes are up-regulated in these plants. In particular, several poplar MYB transcription factor with a C2-repressor motif (LNL[D/E] L-[G/S]) in the C-terminal region were identified. Transient promoter-reporter activation assays in plant cells revealed that one of these repressor-like poplar MYB factors caused a strong repressive effect on promoter activation by the MYB134/bHLH complex. This suggests its involvement in negative feedback regulation of the PA biosynthetic pathway. Expression analysis and mutagenesis suggest that this repressor may be important in stress-induction of PA biosynthesis and act via direct protein-protein interactions.

P-21

CHEMICAL COMPOSITION OF *CALLISTEMON CITRINUS* FROM MEXICO

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Many studies have been done on medicinal properties of different species of *Callistemon*; recently we reported the antibacterial activity of *Callistemon citrinus* growing in Mexico, however there are not reports on the chemical composition. The ethanolic extracts obtained from the leaves of two different ages of *Callistemon citrinus* were analyzed by GC-MS and GC x GC-ToFMS. By applying the first methodology, thirty-two compounds were identified in the old leaves whereas fifty-two compounds in the younger leaves were determined. The second methodology allows identifying three hundred compounds for young leaves and one hundred eighty seven compounds for older leaves. This study reveals high content of terpenoids with the major components: 1,8 cineolo, α -terpinole and α -pinene (monoterpenoids) and spathulenol, viridiflore and β -gurjunene (sesquiterpenoids).

P-22

MOLECULAR DIVERSITY OF TULIPOSIDE A-CONVERTING ENZYME IN TULIP

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Tuliposides, the glucose esters of 4-hydroxy-2-methylenebutanoate and 3,4-dihydroxy-2-methylenebutanoate, are representative secondary metabolites in tulip (*Tulipa gesneriana*). They are converted to the biologically active lactonized aglycons, tulipalins, by tuliposide-converting enzymes. We recently isolated *TgTCEA* gene encoding tuliposide A-converting enzyme (TCEA) from tulip petals, which was the first identified member of the lactone-forming carboxylesterases, specifically catalyzing intramolecular transesterification. *TgTCEA* was transcribed in all tulip tissues but not in bulbs despite higher TCEA activity in bulb extracts than in other tissues, which allowed predicting the presence of a bulb-specific *TgTCEA* isozyme. Here, to prove this hypothesis, the *TgTCEA* homolog, *TgTCEA-b*, was isolated from tulip bulbs. *TgTCEA-b* polypeptides showed approximately 77% identity with petal *TgTCEA*. Functional characterization of *E. coli*-expressed recombinant enzyme verified that *TgTCEA-b* catalyzes the conversion of 6-tuliposide A to tulipalin A, like the petal *TgTCEA* enzyme. Quantitative RT-PCR analysis revealed that *TgTCEA-b* is transcribed efficiently in bulbs unlike *TgTCEA* gene whose transcripts are absent in bulbs, whereas *TgTCEA-b* transcripts were minority in other tissues where *TgTCEA* is transcribed predominantly, showing the tissue preference for *TgTCEA-b* and *TgTCEA* transcription. Moreover, based on subcellular localization of those enzymes, we propose the cytological mechanism of *TgTCEA*-mediated tulipalin formation in the tulip defensive strategy.

P-23

THE NOVEL COMPOUNDS, ISOLIQUIRITIGENIN C-GLYCOSIDE AND MALONYL ISOLIQUIRITIGENIN C-GLYCOSIDE ACCUMULATION IN ISOFLAVONE SYNTHASE RNAi DOWN REGULATED KUDZU (*PUERARIA LOBATA*) HAIRY ROOTS.

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Kudzu (*Pueraria lobata*) is a leguminous plant and is a rich source of isoflavonoids especially the daidzein 8-*C* glycoside, puerarin. *Radix puerariae* (root of kudzu) has long been used as herb medicine in Asia for treatments of fever, hypertension and alcoholism. In recent years, the total isoflavonoid of *Radix puerariae* or the isolated puerarin has been used for treatments of cardio- and cerebral vascular system diseases. Puerarin injection could promote cerebral blood circulation and blood metabolism. Although isoflavone biosynthesis has been clearly defined in molecular biology level puerarin biosynthesis is not well understood especially the isoflavone *C*-glycosylation step. In order to understand puerarin biosynthesis isoflavone synthase (IFS) was down regulated in kudzu hairy root. In IFS down regulated lines all isoflavone metabolites were significantly reduced including puerarin with accumulation of two novel compounds, isoliquiritigenin *C*-glycoside and malonyl isoliquiritigenin *C*-glycoside. Yeast elicitor treatments in IFS down regulated lines showed increased accumulation of malonyl isoliquiritigenin *C*-glycoside, isoliquiritigenin and liquiritigenin. Although it was suggested by labeling study that chalcone isoliquiritigenin might be the precursor for *C*-glycosylation purified isoliquiritigenin *C*-glycoside was not a substrate of recombinant kudzu CHI 1 and 2 in our *in vitro* enzyme reaction. The possible puerarin biosynthesis is discussed.

P-24

DIARYLHEPTANOIDS FROM *ALNUS JAPONICA* INHIBIT PAPAIN-LIKE PROTEASES OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS

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The papain-like protease (PL^{pro}), which controls replication of the severe acute respiratory syndrome (SARS) coronavirus, has been identified as a potential drug target for the treatment of SARS. An intensive hunt for effective anti-SARS drugs has been undertaken by screening for natural product inhibitors that target SARS-CoV PL^{pro}. In this study, diarylheptanoids **1–9** were isolated from *Alnus japonica*, and the inhibitory activities of these compounds against PL^{pro} were determined. Of the isolated diarylheptanoids, hirsutenone (**2**) showed the most potent PL^{pro} inhibitory activity, with an IC₅₀ value of 4.1 μM. Structure-activity analysis showed that catechol and α,β-unsaturated carbonyl moiety in the molecule were the key requirement for SARS-CoV cysteine protease inhibition.

P-25

HETEROLOGOUS EXPRESSION OF ENZYMATIC COCKTAIL COMPONENTS FOR LIGNOCELLULOSIC BIOMASS DECONSTRUCTION

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Plants are responsible for fixing CO₂ into carbohydrates that can be potentially used for biofuel production. However, due to the recalcitrant nature of cell wall carbohydrate polymers (cellulose, hemicellulose, pectin, lignin) and the variability of biomass feedstock availability, the use of a specialized enzymatic cocktail for each feedstock is extremely important for a more efficient decomposition of plant cell wall carbohydrates into fermentable sugars. Using transient expression in *Nicotiana benthamiana* we produced a series of cell wall degrading enzymes from diverse microorganisms targeting each main component of the plant cell wall. The highest expression level achieved was from a lipase gene of *Acremonium alcalophilum*, 9% of total soluble protein (TSP), which breaks down xylan acetyl substrates. The other enzymes, polygalacturonase I from *Aspergillus niger*, laccase from *Trametes vesicolor* and feruloyl esterase from *Anaeromyces mucronatus* had their highest expression levels at 3.5, 2 and 4% of TSP respectively. Xylanase A and the cellulose-degrading enzymes endoglucanase H and exoglucanase S from *Clostridium cellulovorans* expressed in tobacco chloroplasts accumulate at levels of 0.8, 0.4 and 0.8% of TSP, respectively. Dosage analysis will be used in the design of enzymatic cocktails for the diverse biomass feedstocks available.

P-26

ISOLATION OF FULL-LENGTH COMPLEMENTARY DNA OF CAFFEIC ACID O-METHYLTRANSFERASE FROM 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, white poplar and whitewood. 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. Previous research demonstrated that when two-year-old yellow poplar was treated with one of the plant steroidal hormone, brassinolide, lignin deposition was significantly reduced and its monomeric composition was modified. In order to understand BR-induced modification of lignin biosynthesis, partial sequence of five COMTs were obtained from the EST database that has been constructed from the tension wood of two-year-old yellow poplar. For functional characterization of yellow poplar, full-length cDNA of five COMTs were isolated and transformed into *E.coli*, Arabidopsis and hybrid poplar. In addition, promoter region of one COMT, whose transcript was abundant in the stem than leaf and was BR-inducible, was isolated using genome working technique.

P-27

THE BIOGEOGRAPHICAL DISTRIBUTION OF DUNCECAP LARKSPUR (*Delphinium occidentale*) CHEMOTYPES AND THEIR POTENTIAL TOXICITY

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Larkspurs (*Delphinium* spp.) are poisonous plants found on rangelands in Western North America. Larkspur's toxicity has been attributed to the norditerpenoid alkaloids which are divided into two main structural groups; the highly toxic (N-methylsuccinimido) anthranoyllycoctonine type (MSAL-type) and the less toxic 7,8-methylenedioxylycoctonine type (MDL-type). Plants high in the MSAL-type alkaloids are thought to be the most toxic to cattle and the concentrations of these alkaloids have been used as a predictor of plant toxicity. Duncecap larkspur, *Delphinium occidentale*, occurs throughout much of the Intermountain West and Northwestern United States. Specimens from field collections and herbaria deposits were evaluated taxonomically and chemically. Two distinct alkaloid profiles were identified: one that contains the MSAL-type alkaloids and one that contains very little, if any, MSAL-type alkaloids. Each alkaloid profile was unique in its geographical distribution. To determine toxic potential, cattle were orally dosed with plant material from two populations representing the two chemotypes to determine the dose, based upon total alkaloid concentration, required for each population to elicit similar clinical signs of poisoning in cattle. The results from this study indicate that the two chemotypes are different in their toxic potential.

P-28: Withdrawn

P-29

***NELUMBO NUCIFERA* SEMEN EXTRACT AMILIORATES SCOPOLAMINE-INDUCED LEARNING AND MEMORY IMPAIRMENT IN MICE**

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Nelumbo nucifera semen is a traditional herb with anti-diarrheal, anti-ganacratia, and tranquilizer like pharmacological activities. The major purpose of this study was to determine the effect of *Nelumbo nucifera* semen extract (NNSE) on the learning and memory impairment induced by scopolamine (1mg/kg, s.c.) in mice. The capacities of memory and learning were evaluated by Morris Water Maze test. NNSE (3, 10, 30, 100, 200mg/kg BW, p.o.) significantly revised scopolamine-induced learning and memory impairment in the Morris Water Maze test, as evaluated by shortened escape latency and swimming distance. In addition, NNSE was also found to inhibit acetylcholinesterase (AChE) activity. These results of study indicate that NNSE may play a useful role in the treatment of cognitive impairment caused by Alzheimer's disease and aging.

P-30

***IN-VIVO AND IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF *DRYNARIA QUERCIFOLIA* FRONDS AGAINST CARBON-TETRACHLORIDE INDUCED HEPATOCELLULAR DAMAGE**

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The present study was conducted to evaluate the hepatoprotective effect of hydroalcoholic extract of *Drynaria quercifolia* fronds (DqE), its fractions (chloroform, ethyl acetate & n-butanol) and isolated active compound (Dq4) from ethyl acetate fraction (Eaf). The toxicant CCl₄ (1 ml/kg in olive oil; *sc*) was administered on 4th and 5th day to induce hepatotoxicity in rats. *In-vitro* hepatoprotection of the test drugs and silymarin was evaluated against CCl₄ (1%) induced toxicity in HepG2 cells. The cytotoxicity of HepG2 cells was estimated by MTT reduction assay and trypan blue dye exclusion method. The pre-treatment of rats with DqE, Eaf, Dq4 and standard drug (silymarin) for 7 days produced a significant dose dependent hepatoprotective action by decreased levels of AST, ALT, ALP, TB and TBARS and increased levels of TP, ALB and reduced GSH. The histological examination provided the supportive evidences with normal parenchymal architecture, absence of centrilobular necrosis, less ballooning degeneration and no noticeable alterations of portal tracts and central veins. Additionally, DqE, Eaf, Dq4 and silymarin significantly decreased the CCl₄-induced toxicity in HepG2 cells. The present study scientifically validated the traditional use of *D. quercifolia* for liver disorders and strongly demonstrates its antioxidative effect on hepatocytes and restoring their normal functional ability.

P-31

ANXIOLYTIC EFFECT OF METHANOL EXTRACT AND ALKALOIDAL FRACTION OF *NELUMBO NUCIFERA* PLUMULES IN MICE

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The objective of the present study was to evaluate the anxiolytic effect of methanolic extract (ME) and alkaloidal fraction (AF) prepared from the plumules of *Nelumbo nucifera* using elevated plus-maze paradigm (EPM) and open field test (OFT). The results were compared with standard drug benzodiazepine diazepam (2 mg/kg p.o.). In the EPM, the ME (50, 100 & 200 mg/kg p.o.) and AF (13.6 mg/kg p.o and 27.4mg/kg p.o.) significantly increased the duration of exploration in open arm *via* time-spent in and the entries into the open arm by mice as compared with normal control. Further, in the OFT, the ME and AF significantly increased the exploratory behaviour of mice by increasing the number of rearings, assisted rearings and number of square traversed as compared with the normal control. The result indicates that *Nelumbo nucifera* is an effective anxiolytic agent which is due to the presence of high concentration of alkaloidal content. The present study provides a scientific evidence for the traditional claim attributed to this plant. Further, investigations are underway to isolate and identify the active constituents from the AF, responsible for the anxiolytic potential.

P-32

TRANSCRIPTOME AND STIMULATORY EFFECT OF PHYTOCHEMICAL SHIKONIN ON THE EPITHELIAL-MESENCHYMAL TRANSITION (EMT) ACTIVITY IN MOUSE SKIN

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Various pharmacological activities of shikonins have been well documented; information on possible genomic and hierarchical regulation for a spectrum of coordinated or integrated bio-activities of shikonins has been very limited. Through cross-examination between transcriptome and microRNA data sets, we hypothesized that shikonin treatment may affect the epithelial-mesenchymal transition (EMT) process and the expression of related microRNAs, including 200a, 200b, 200c, 141, 205 and 429 microRNAs, in test skin tissues. Subsequent *in situ* tests on mouse abdominal skin tissues confirmed the stimulatory effect of shikonin on specific regulatory molecules of the EMT process in test epidermal tissue. RT-PCR analyses then confirmed the downregulating effects of shikonin on expression of microRNA 205 and members of the microRNA 200 family known as involved in EMT process. Gene expression of two RNA targets of the microRNA 200 family in EMT regulation, namely *Sip1 (Zeb2)* and *Tcf8 (Zeb1)*, were consistently up-regulated by shikonin treatment. Our findings suggest that topical treatment with shikonin can confer a potent stimulatory effect on the EMT process and suppress the associated-microRNAs expression *in vivo* in skin tissues. These *in vivo* data provide good cellular and molecular evidence in support of our previous findings on specific pharmacological effects of shikonin, including wound-healing and immune-modulatory activities.

P-33

PHYTOCHEMICALS FROM *ALLIUM FISTULOSUM* L. EXTRACT EFFECTIVELY INHIBIT COLORECTAL TUMOR GROWTH: CELLULAR MECHANISMS AND NUTRITIONAL APPLICATIONS

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Allium fistulosum L., also commonly known as scallion, is used as a spice or vegetable worldwide. The possible beneficial effects of scallion on mouse colon cancer are evaluated in this study. The *in vivo* effects of scallion extracts were assessed in a subcutaneously inoculated CT-26 colon tumor model in BALB/c mice. Tumor tissue sections were subjected to western blotting for analysis of key inflammatory markers, ELISA for analysis of cytokines, and immunohistochemistry for analysis of inflammatory markers. Scallion extracts, particularly the hot-water extract, orally fed to mice at 50 mg (dry weight)/kg body weight resulted in a significantly suppression of tumor growth and enhanced the survival rate of test mice. At the level of molecular signaling/networking activities, scallion extracts inhibited the key inflammatory markers COX-2 and iNOS, and suppressed the expression of various cellular markers known to be involved in tumor apoptosis, proliferation (cyclin D1 and c-Myc), angiogenesis (VEGF and HIF-1 α), and tumor invasion (MMP-9 and ICAM-1), when compared with vehicle control-treated mice. Our findings may warrant further investigation for the use of common scallion as a chemopreventive dietary agent to lower the risk of colon cancer.

P-34

TUMOR SUPPRESSIVE EFFECT OF HERBAL EXTRACTS FROM TRADITIONAL MEDICINAL PLANTS ON CYTOKINE REGULATION, PRIMARY TUMOR GROWTH AND METASTASIS IN ORTHOTOPIC MAMMARY TUMOR MODEL

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Traditional medicinal plants (TMP) are increasingly recognized for use in public health care throughout the world. However, systematic investigation of TMPs on specific and health care-applicable immuno-regulatory activities is limited. Dendritic cells (DCs), a key type of professional antigen presenting cells are key mediators in human's immune systems. Therefore, DCs are considered by many as a viable pharmacological platform or target for evaluating TMP's immuno-regulatory activities. The objective of this study is to evaluate the regulatory activities of specific TMPs on cytokine regulation in DCs and anti-tumor activities in orthotopic mammary tumor model. Our results show that a number of extracts of test Taiwan specialty medicinal herbs exhibited an inhibitory activity on LPS-induced expression of IL-6 and IL-12 in mouse DCs. In addition, these herbal extracts were categorized into several functional sub-groups based on their capacities to regulate cytokine expression. In an orthotopic mammary tumor model, the extract of TMP1 effectively suppressed primary tumor growth. The extracts of both TMP1 and TMP2 suppressed mammary tumor metastasis, and the results were further confirmed by a mammary tumor resection model. Taken together, our results suggest that TMP1 and TMP2 may warrant further investigation for potential application as anti-tumor natural product agents.

P-35

EFFECT OF PtMYB134 AND PtMYB97 TRANSCRIPTION FACTOR OVEREXPRESSION ON FLAVONOIDS AND PHENOLIC GLYCOSIDES IN HYBRID POPLAR

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Poplar trees (*Populus* spp) are well known to produce a large range of phenolic secondary metabolites, including proanthocyanidins (condensed tannins), phenolic glycosides, and hydroxycinnamate esters. These pathways are believed to be regulated by MYB R2R3 transcription factors, proteins involved in the regulation of many biosynthetic pathways in plants. In this study we assess the phenolic profiles of different transgenic poplar lines and genotypes including *Populus tremula* x *P. alba* and *P. tremula* x *P. tremuloides* overexpressing the poplar MYB134 and MYB97 transcription factors. Plants samples were harvested (LPI 1, LPI 5, LPI 10, phloem and xylem between each harvested leaves, old and young roots) and analyzed by HPLC, HPLC/MS/MS for their phenolic content. We found that some transgenic lines demonstrate interesting variation in secondary metabolite content, but that this varied depending on the plant tissue. MYB134 was previously characterized and shown to regulate the proanthocyanidin pathway (Mellway *et al.*, Plant Physiol 150: 924-941, 2009), but the function of MYB097 is not known. Ultimately, we plan to compare the transcriptional profiles with this metabolomics analysis in order to identify novel genes involved in the biosynthesis of phenolics.

P-36

VIGNA ANGULARIS INHIBITS IL-6-INDUCED CELLULAR SIGNALING AND AMELIORATES COLLAGEN-INDUCED ARTHRITIS

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Vigna angularis (azuki bean) is reported to possess various medicinal effects, such as tumor-suppressive, renal-protective, anti-diabetic, anti-oxidative, and anti-inflammatory effects. However, little is known about the molecular mechanisms of the medicinal effects of azuki bean. In this study, we investigated the possible mechanisms underlying the effects of *V. angularis* (VAE). We demonstrated for the first time that extracts or isolated triterpenoid compounds from *V. angularis* were able to suppress IL-6 signaling, from Janus kinases to STAT3 protein phosphorylation and transcriptional activation of STAT3. It also substantially inhibited the expression of C-reactive protein (CRP), a marker of inflammation and cancer, induced by STAT3. Since IL-6 plays a crucial role in human autoimmune diseases including rheumatoid arthritis (RA), we examined the effect of VAE on the development of collagen-induced arthritis (CIA). Indeed, VAE effectively ameliorated the development of CIA, accompanied by a reduced antibody response to type II collagen and the absence of tissue damage in knee joints. These results provide an interpretable mechanism for the medicinal effects of *V. angularis* and suggest that extract or compounds from *V. angularis* could be useful remedy for treatment of wide spectrum of diseases related to IL-6.

P-37

COGNITIVE ENHANCING ACTIVITY OF *CODONOPSIS LANCEOLATA* WITH STEAMING PROCESS ON SCOPOLAMINE-INDUCED IMPAIRMENT ON MICE

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Codonopsis lanceolata (Campanulaceae) traditionally have been used as tonic and for treatment of lung abscesses. The purpose of this study was to evaluate the cognitive enhancing effect of steamed *C. lanceolata* in scopolamine-induced memory impairment mice using by passive avoidance test and Morris water maze tests. *C. lanceolata* extract was orally administered to male mice at the doses of 100, 300 and 500 mg/kg body weight. *C. lanceolata* extract (300 mg/kg body weight, p.o.) treated group showed shorter escape latencies than the scopolamine-administered group in Morris water maze test. Also, it exerted longer step-through latency time than scopolamine treated group in passive avoidance test. As the results, steamed *C. lanceolata* extract showed cognitive-enhancing activities related to the memory processes. Further study will be required to investigate the mechanism of cognitive-enhancing activity.

P-38

**NEUROPROTECTIVE EFFECT OF HOMOSYRINGALDEHYDE ISOLATED FROM
*CYNANCHUM PANICULATUM***

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Homosyringaldehyde was isolated and identified from the 80% methanol extract of roots of *Cynanchum paniculatum*. *C. paniculatum* has been widely used for the treatment of various diseases such as neurasthenia, insomnia, dysmenorrheal and toothache. This compound exerted significant neuroprotective activities against glutamate-induced neurotoxicity in hippocampal HT22 cell line by 37.53 % (at the concentration of 100 μ M). We investigated mode of action of this compound. Homosyringaldehyde (100 μ M) significantly decreased the ROS level in the oxidative stress induced HT22 cells by glutamate excitotoxicity. Thus, our results suggest that homosyringaldehyde significantly protect HT22 cells against glutamate-induced oxidative stress, via antioxidative activities. As the results, we suggest that homosyringaldehyde may be useful in the treatment of neurodegenerative disorders.

P-39

**SIMULTANEOUS DETERMINATION OF NINE MAJOR COMPONENTS IN
TRADITIONAL HERBAL MEDICINE 'PALMUL-TNAG' BY HPLC-DAD**

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Palmul-tang is a traditional herbal medicine, is composed of eight herbs (Ginseng Radix Alba, Glycyrrhizae Radix, Hoelen, Atractylodis Rhizoma, Angelicae Gigantis Radix, Cnidii Rhizoma, Paeoniae Radix, and Rehmanniae Radix preparata). Previous investigations reported that Palmul-tang exhibit various bioactivities, including anti-allergic effect, anti-inflammatory, antioxidative effect and antitumor effect. In this study, an effective, reliable and accurate high performance liquid chromatography method has been developed for the determination of nine major components in Palmul-tang. All the calibration curves of the nine components indicated excellent linearity ($r^2 > 0.9997$) within the test range. The limit of detection (LOD) and limit of quantification (LOQ) of each component were in the ranges 0.08~1.03 μ g/mL and 0.23~3.11 μ g/mL, respectively. The intra day and inter day precision were within 1.65% and 2.71%, respectively. Finally, the established analytical method had good accuracy, with a recovery of 94.49~101.10% for all the assayed analytes. The established method was successfully applied to the simultaneous determination of the nine major components in 12 samples of Palmul-tang. The analytical method might be very simple and suitable for the quality control of Palmul-tang.

P-40

PARADOXICAL IMMUNO-MODULATING EFFECT OF NORTH AMERICAN GINSENG AQUEOUS AND POLYSACCHARIDE EXTRACTS ON MACROPHAGE IMMUNE FUNCTION

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The paradoxical immuno-modulatory effect of North American (NA) ginseng aqueous (AQ) and crude polysaccharide (PS) extracts was examined in this study. Production of pro-inflammatory mediators in culture by alveolar macrophages isolated from adult rats treated orally with 125mg/Kg AQ extract for 3 or 6 days was used to evaluate the influence of ginseng on macrophage immune function. Both treatments when compared to untreated control group resulted in significant increase in pro-inflammatory mediator production. Immuno-stimulatory effect was more in *in vitro* study probably due to lower bioavailability of the orally-administered extracts. The 6 days was more potent than the 3 days AQ and PS extract treatments in suppressing LPS infectious-inflammatory response in culture. This *in vivo* paradoxical effect was replicated in *in vitro* study which showed similar anti-inflammatory effect (desensitization of LPS inflammatory response) in alveolar macrophages by pre-treatment with AQ or crude PS extract and its acidic PS fraction for 24 hr prior to LPS challenge. Acidic PS but not neutral PS was responsible for *in vitro* paradoxical immuno-modulatory effect of NA ginseng crude polysaccharide and aqueous extracts. Outcome of this study suggest acidic PS as the bioactive which mediates the paradoxical immuno-modulatory effect of crude PS and AQ extracts.

P-41

BIOLOGICAL ACTIVE SECONDARY METABOLITES FROM *ASPODELUS MICROCARPUS SALZM. ET VIVI*

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Bioassay guided fractionation of the methanolic extract of *Aspodelus microcarpus Salzm. et Vivi* (Asphodelaceae) resulted in the isolation of seven compounds. Six known compounds were identified as 1,1',8,8'-tetrahydroxy-3,3'-dimethyl-4,7'-bianthraquinoyl (Asphodelin); 1,8-dihydroxy-3-methyl anthracendione (Chrysophanol); 8-methoxy chrysophanol; 2-acetyl,1,8-dimethoxy,3-methyl naphthalene; 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone; 4,9-dihydroxy-8-methoxy-6-methyl-3,4-dihydroanthracen-1[2H]-one (Aloesaponol III -8-methyl ether) and a new compound: 3,10-dimethoxy-5-methyl-1H-1,4 epoxybenzo[h]isochromen, by 1D and 2D NMR as well as HERMS. Compounds chrysophanol and 8-methoxy chrysophanol showed good to moderate antileishmanial activity with an IC₅₀ values of 14.3 and 35.17 µg/ml respectively. Compound 8-methoxy chrysophanol exhibited moderate antifungal activity against *Cryptococcus neoformans* with an IC₅₀ 15 µg/ml, while compound 2-acetyl,1,8-dimethoxy,3-methyl naphthalene showed good activity against methicillin resistant *Staphylococcus aureus* (MRSA) with an IC₅₀ 9.43 µg/ml and moderate activity against leukemia K562 cells (58% inhibition).

P-42

ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL ACTIVITY OF SECONDARY METABOLITES FROM THE ENDOPHYTIC FUNGUS *NIGROSPORA SAPHERICA*

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The endophytic fungus *Nigrospora sapherica* was isolated from *Vinca rosea* leaves growing in Egypt. The fungus was grown on solid media (Asian rice). Its Ethyl acetate extract was subjected to liquid/liquid fractionation using hexanes, methanol, butanol and water. The butanol and water fractions displayed antimalarial activities against *Plasmodium falsiparum* D6 clone with IC₅₀ values of 9.7 and 17.0 µg/mL, respectively, and W2 clone with IC₅₀ values of 9.3 and 20.0 µg/mL, respectively. Seven compounds including five sterols and two furan derivatives have been isolated from *N. sapherica* for the first time. The isolated compounds were chemically identified as ergosta-6,22-diene-3β,5α,8α-triol, ergosta-7,22-diene-3-ol, ergosta-4,6,8 (14),22-tetraene-3β-ol, ergosta-4,6,8 (14),22 tetraene-3-one, ergosta-5,7,22-triene-3β-ol, 2(3H)-furanone, dihydro-4-(hydroxyl methyl)-3,5-dimethyl and its isomer using 1D NMR (¹H, ¹³C, DEPT135) and 2D NMR (COSY, NOESY, HMQC and HMBC) as well as HR-ESI-MS. Five Fatty acids have been also isolated and chemically identified as stearic acid, oleic acid, palmitic acid, myristic acid, and sebacic acid dibutyl ester using GC/MS. The antimalarial, antileishmanial, and antimicrobial activities of the isolates were investigated.

P-43

NEW ANTHRAQUINONE DERIVATIVES FROM *GEOSMITHIA LAVENDULA*

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Geosmithia lavendula Pitt (Acomycota: Hypocreales) provided by Assiut University Mycological Center (Accession No. 1004) is a lilac colored filamentous fungus living in symbiosis with bark beetles. It was reported that hydroxylated anthraquinones were found to be the most abundant compounds produced into the medium during the submerged cultivation. When the fungus was grown on MID liquid media, no anthraquinones were detected or isolated from this media. Six compounds were isolated and identified as *p*-hydroxy benzyl alcohol, palmitic acid, oleic acid, mannitol, (22*E*)-ergosta-6,22-diene-3β,5α,8α-triol and acetamide-2-hydroxy-*N*-tetradecyl. The latter being a newly discovered natural product. When the fungus was grown on Potato Dextrose Broth (PDB), anthraquinones were detected in the media. Four main anthraquinones were isolated and identified as 1-acetyl-2,4,5,7- tetrahydroxy-9,10 anthraquinone, 1-acetyl-2,4,5,7,8- pentahydroxy-9,10 anthraquinone, 1-acetyl-2,4,6,8- tetrahydroxy-9,10 anthraquinone and 2-acetyl-1,4,5,7- tetrahydroxy-9,10 anthraquinone. The last two are new naturally isolated and structurally elucidated anthraquinones. The isolated compounds were subjected to antibacterial, antifungal, antimalarial and antileishmanial assays. 1-acetyl-2,4,6,8- tetrahydroxy-9,10 anthraquinone was found to have moderate activity against methicillin resistant *Staphylococcus aureus* with an IC₅₀ value of 16.12 µg/ml.

P-44

TRANSCRIPTIONAL AND METABOLIC CHANGES IN ARABIDOPSIS ROOTS IN RESPONSE TO HERBIVORY

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Plant defense against belowground herbivory is essential to maintain efficient uptake of water and nutrients. Despite growing evidence of root-specific chemical defenses against herbivores and the interaction between herbivore-induced above and belowground defense systems, a comprehensive understanding of the metabolic, biochemical, and molecular responses of roots to herbivore attack is missing. We have developed an *Arabidopsis thaliana* aeroponic culture system that allows the generalist root herbivore *Bradysia* (fungus gnat) to feed on plant roots in a soil-like environment. A preliminary microarray analysis suggests substantial modifications in amino acid metabolism and nitrogen/carbon resource mobilization in roots with possible consequences in nutrient deprivation for the herbivore. We have found an upregulation of genes that are normally induced under energy stress in leaves and associated with protein degradation, the formation of asparagine, and the conversion of essential amino acids including threonine and branched chain amino acids (BCAAs). For example, the expression of *Threonine Aldolase 1* (*THA1*), which is involved with converting threonine to glycine in *Arabidopsis* seeds, was induced 5-fold (log₂ scale) upon four days of insect feeding has been detected 30-fold upregulated in roots after feeding for four days. Interestingly, *THA1* has been identified primarily expressing in seeds and seedlings. Together, the results suggest a reprogramming of root metabolism with reallocation of nitrogen and carbon resources between below- and aboveground tissues.

P-45

QUERCETIN 3-O-GLUCOSYL HYDROLASE ACTIVITY IN ARABIDOPSIS DURING RECOVERY FROM ABIOTIC STRESS

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Quercetin 3-*O*-glucosyl-7-*O*-rhamnoside (Q3G7R), a major flavonol diglycoside, accumulates in *Arabidopsis thaliana* plants during a combined abiotic stress (i.e. nitrogen deficiency and low temperature; NDLT). However, the level of Q3G7R is significantly reduced within 2 days of removing the stress. The biochemical mechanisms governing flavonol catabolism are not well known. To determine if the loss of Q3G7R is associated with its enzymatic hydrolysis during recovery from the stress, we assayed flavonol 3-*O*-glucosyl hydrolase (F3GH) activity of NDLT-treated *Arabidopsis* following the coordinated re-supply of nitrate (10 mM) and return to ambient temperature (21°C). An HPLC-based assay was developed for quantification of F3GH activity. F3GH activity of cell-free extracts (in the presence of Q3G7R) increased by 222% within 2 days of stress removal. The product of the Q3G7R hydrolysis assay was quercetin 7-*O*-rhamnoside, as identified by quadrupole time-of-flight mass spectrometry. Also, in assays utilizing quercetin 3-*O*-glucoside as the substrate, a 5.2-fold increase in F3GH activity was apparent within 3 days of stress removal. These data indicate that two independent F3GH activities are induced in *Arabidopsis* during NDLT recovery, which may represent distinct catabolic routes for the loss of Q3G7R. Transcript profiling of putative F3GHs is currently underway.

P-46

APOCAROTENOID EMISSION STUDY IN WILD TYPE AND TRANSGENIC *ARABIDOPSIS* PLANTS OVER-EXPRESSING *CCD-1* AND THEIR FEEDING EFFECTS AGAINST CABBAGE LOOPERS

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The oxidative cleavage of carotenoids, catalyzed by a family of carotenoid cleavage dioxygenases (CCDs), leads to the production of apocarotenoids. In this study, transgenic *Arabidopsis* plants over-expressing a *CCD1* gene were generated to test whether or not production of volatiles such as alpha and beta ionone, theaspirane A and B, and damascone could be enhanced to control feeding by cabbage loopers (*Trichoplusia ni*). Genetically transformed *Arabidopsis* plants are morphologically indistinguishable from the wild type (WT) at any development stage, but gas chromatography-mass spectrometry analysis of headspace volatiles collected from 6-wk-old intact flowering plants revealed differences in profiles of volatile metabolites emitted by the plants. Dynamic head-space collection of volatiles using Porapak-Q cartridges revealed substantially enhanced ionone, theaspirane A and B, and damascone emissions from transgenic plants compared with WT. Eight transgenic and eight WT *Arabidopsis* plants were exposed to 60 2nd instar cabbage loopers during a 24 h period. Results in terms of consumption by the insects showed that 13.03% of WT leaves were eaten compared to 25.20% of CCD1 leaves. Bioassays using 2nd instar cabbage loopers exposed to standards of the volatile molecules collected from in CCD1 plants will be performed in order to characterize insect behavior.

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METABOLISM OF THE SOYBEAN-FUNGUS HOST-PATHOGEN INTERACTION – A PLANT METABOLOMICS STUDY

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The destructive soilborne pathogens of soybean [*Glycine max* (L.) Merr.], *Phytophthora sojae*, *Pythium ultimum*, *Fusarium solani* f.sp. and *Rhizoctonia solani*, cause significant yield reductions worldwide, but currently there is no rapid or commercially available test to identify each disease using unique metabolic biomarkers. Such information would greatly improve the ability of growers to make management decisions to minimize disease damage to soybean crops. Here we report a GC-MS metabolomic study to investigate metabolites of infected and uninfected plants as well as mycelium. Metabolic differences were found between soybean plants infected with the oomycetes (*P. sojae* and *P. ultimum*) and fungi (*F. solani* and *R. solani*). The phytoalexins glyceollin I and glyceollin II were found in soybean infected with all four diseases, but glyceollin III was produced in soybean only by *F. solani* and *R. solani*. The amount of sitosterol and sugars decreased in infected compared with control plants for all four diseases. *P. ultimum* produced 3,9-Dioxa-2,10-disilaundecane, a unique plant metabolite biomarker in infected plants, and two unique pathogen metabolites were also observed, eicosapentaenoic acid and arachidonic acid. *F. solani*, produced 15-acetoxyscirpenol and three as yet unidentified biomarkers. *P. sojae* and *R. solani* each produced one as yet unidentified unique metabolite.

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GINSENOSES AND THE PATHOGENICITY OF *PYTHIUM IRREGULARE*

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American ginseng (*Panax quinquefolius* L.) produces triterpenoid saponins, ginsenosides, that possess mild fungitoxic activity toward some common ginseng leaf pathogens. However, they also enhance the growth of some pathogens, most notably the oomycete *Pythium irregulare* Buisman, which is able to partially deglycosylate the 20 (*S*)-protopanaxadiol ginsenosides Rb1, Rd and gypenoside XVII via extracellular glycosidases (ginsenosidases), leading to a common product, ginsenoside F2. Previously, it has been shown that the ability of nine distinct isolates of *P. irregulare* to deglycosylate 20 (*S*)-protopanaxadiols *in vitro* was correlated to their pathogenicity toward one- and two-year old ginseng seedlings. Since the appearance of ginsenosidases in the culture medium of *P. irregulare* is dependent on exposure to ginsenosides, we hypothesized that ginsenosidases help *Pythium* find its host and/or obtain nutrients/growth factors from the environment. Furthermore, it was believed that ginsenoside F2 could act as a host recognition factor for *P. irregulare*, facilitating the production of ginsenosidases and up-regulating the growth of the organism. However, in depth analysis has shown that exposure of *P. irregulare* to partially purified ginsenoside F2 *in vitro* leads to inhibition of growth, even though increased growth is observed when *Pythium* was exposed to mixed ginsenosides.

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CHARACTERIZATION OF REGIOSELECTIVE FLAVONE *O*-METHYLTRANSFERASES FROM SWEET BASIL.

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Sweet basil (*Ocimum basilicum* L.) is best known as a popular culinary herb. Alongside the volatile compounds lending basil leaves their much prized fragrance, lipophilic flavones characterized by hydroxylations at positions 6 and 8 and subsequent methylations also accumulate. The metabolic network underlying the accumulation of these flavones has not been studied to date. We verified that flavones are predominantly localized in peltate glandular trichomes in basil, and used our trichome-specific transcriptome database to identify putative flavone *O*-methyltransferases (OMTs) that could engender 6-, 7-, and 4'-*O*-methylations necessary to produce salvigenin, one of the most abundant flavones in basil. Six recombinant proteins whose activities are expected to cover the diversity of major OMTs present in the EST database were purified and characterized. While flavone 7-OMTs displayed strict regioselectivity, the putative flavone 4'-OMTs could catalyze both 4'- and 6-*O*-methylations. Only a few amino acid changes were necessary to switch the regioselectivity of a 4'-OMT into that of a 6-OMT. Kinetic data indicated all OMTs to be highly selective for only few substrates. Combined biochemical data has allowed us to outline some central routes and key intermediates of salvigenin production in basil.

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ENZYMATIC SYNTHESIS OF PUERARIN GLUCOSIDES USING LEUCONOSTOC DEXTRANSUCRASE

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Puerarin (P), an isoflavone derived from kudzu roots, has strong biological activities, but its bioavailability is often limited by its low water solubility. To increase its solubility, P was glucosylated by three dextransucrases from *Leuconostoc* or *Streptococcus* species. *Leuconostoc lactis* EG001 dextransucrase exhibited the highest productivity of puerarin glucosides (P-Gs) among the three tested enzymes, and it primarily produced two P-Gs with a 53% yield. Their structures were identified as alpha-D-glucosyl-(1-->6)-P (P-G) by using LC-MS or ¹H- or ¹³C-NMR spectroscopies and alpha-D-isomaltosyl-(1-->6)-P (P-IG2) by using specific enzymatic hydrolysis, and their solubilities were 15- and 202-fold higher than that of P, respectively. P-G and P-IG2 are easily applicable in the food and pharmaceutical industries as alternative functional materials.

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