Biologically Active Phytochemicals

August 8-12, 2009
Towson University
Towson, Maryland

49th Annual Phytochemical Society of North America Meeting and Symposia

Phytochemical Society of North America

49th Annual Meeting

and

Symposia

Biologically Active Phytochemicals

August 8 – 12, 2009

On the Campus of

Towson University

Towson, Maryland

Conference and Symposia Organizers

James A. Saunders, Towson University, Chair Organizing Committee
Benjamin Matthews, USDA
Nadim Alkharouf, Towson University
Jed Fahey, Johns Hopkins University
Mark A. Bernards, University of Western Ontario
Erin Young, Towson University
Tim Martin, Towson University
Christina Engstrom, Towson University
Tissa Thomas, Towson University
Christopher Saunders, Towson University

August 8, 2009 Saturday

PSNA Executive Committee meeting	3:00 - 5:00 PM	RM 360 Smith Hall
Registration and Arrival	2:00 – 8:00 PM	Chesapeake Rooms Stud
Student Union		
Key Note Presentation		
James Duke	6:00 – 7:00 PM	
The Herbal Village		
Chesapeake Rooms, Student Union		
Spices and Culinary Herbs in Folk Medicine and Phytomedicine		
Reception	7:00 – 8:30 PM	Chesapeake Rooms Student Union

August 9, 2009 Sunday

Session I: Moderator James A. Saunders, Towson University

Chao Lu		
Dean Graduate School, Towson University		
Opening Remarks	8:30 – 9:00 AM	Lecture Room 326 Smith Hall
Jed Fahey	9:00 – 9:45 AM	Lecture Room 326 Smith Hall
Johns Hopkins University		
The action of glucosinolates / isothiocyanates on *Helicobacter pylori*		
A. Douglas Kinghorn	9:45 – 10:30 AM	Lecture Room 326 Smith Hall
Ohio State University		
Discovery of Potential Anticancer Agents from Tropical Plants		
Morning Break	10:30 – 11:00 AM	
Eloy Rodriguez	11:00– 11:20 AM	Lecture Room 326 Smith Hall
Cornell University		
The Biochemical Evolution of Phytomedicines		
Zhonghua Wang, Hong Han, Reinhard Jetter	11:20 – 11:40 AM	Lecture Room 326 Smith Hall
University of British Columbia		
Isolation and Characterization of the *Kalanchoe daigremontiana* Cyclases forming the Cuticular Triterpenoids Glutinol and Friedelin		

Cedric B. Baker Mercer University-College of Pharmacy and Health Sciences **Bioactive Phytochemicals in Medical Foods for Phytotherapy**	11:40-12:00 PM	Lecture Room 326 Smith Hall
Lunch	12:00 – 1:05 PM	Chesapeake Rooms Student Union

Session II: Moderator Cecilia McIntosh, East Tennessee State University

Jinhui Dou Food and Drug Administration **Botanical Drug Development in the U.S.**	1:10 - 1:55 PM	Lecture Room 326 Smith Hall
Poster Introductions (2 minute rounds)	1:55 – 3:15 PM	Lecture Room 326 Smith Hall
Poster Session and refreshments Student Union	3:15 – 5:00 PM	Chesapeake Rooms

August 10, 2009 Monday

Session III: Moderator Roland Roberts, Towson University

Jeffrey Weidenhamer 8:45 – 9:30 AM Lecture Room 326
Ashland University Smith Hall
Analytical Strategies for Ecologically Active Phytochemicals

Vonnie Shields 9:30 – 10:15 AM Lecture Room 326
Towson University Smith Hall
The Effects of Alkaloids on the Feeding Behavior and Neurophysiology of Insects

Morning Break 10:15 – 10:45 AM

Neish Speaker
Gale G. Bozzo 10:45 – 11:15 AM Lecture Room 326
University of Guelph Smith Hall
Salvage of Folate Catabolites in Plants: Biochemical Characterization of *p*-Aminobenzoylglutamate Hydrolase

Neish Speaker
Syed G.A. Moinuddin, Dhrubojyoti D. Laskar, Chanyoung Ki, Laurence B. Davin, Norman G. Lewis 11:15 – 11:45 AM Lecture Room 326
Washington State University Smith Hall
COMT (CAFFEIC ACID *O*-METHYL TRANSFERASE) Mutations and Effects on Both Lignin Primary Structure and Deconstruction

Lunch 11:50 – 1:00 PM Chesapeake Rooms
Student Union

Session IV: Moderator Tim Dwyer, Stevenson University

Xing Cong Li 1:10 – 1:55 PM Lecture Room 326
University of Mississippi Smith Hall
Drug Discovery of Natural Products for Pharmacognosy

Cristobal L. Miranda and Jan F. Stevens 1:55 – 2:15 PM Lecture Room 326
Oregon State University Smith Hall

Xanthohumol and Related Prenylated Flavonoids from Hops Inhibit Production of Inflammatory Mediators in Activated Monocytes and Macrophages

Daniel K. Owens and Cecilia A. McIntosh 2:15 – 2:35 PM Lecture Room 326
East Tennessee State University Smith Hall

Identification, Biochemical Characterization, and Structure Function Analysis of a Flavonol 3-O-Glucosyltransferase from Citrus Paradisi

Ming-Zhu Shi, Matthew Gromlich, Li-Li Zhou, De-Yu Xie
North Carolina State University 2:35 – 2:55 PM Lecture Room 326
Smith Hall

Red Callus Culture of Arabidopsis as a Tool for Analyzing the Regulation Mechanism Controlling the Function of Pap1 Transcription Factor

Poster Session and Refreshments 3:00 – 4:00 PM Chesapeake Rooms
Student Union

PSNA Business meeting, 4:00 – 5:00 PM Chesapeake Rooms
Student Union

August 11, 2009 Tuesday

Session V: Moderator John Thor Arnason, University of Ottawa

Bill J. Baker 8:45 – 9:30 AM Lecture Room 326
University of South Florida Smith Hall
Antarctic Red Marine Algae Produces Influenza Inhibitory Chemistry

Michael C. Tims 9:30 – 10:15 AM Lecture Room 326
National Institutes Standards & Technology Smith Hall
A Little Biology Goes a Long Way: Evaluation of Extraction Methods for Gallic Acid, Catechins and Xanthines in Green Tea

Morning Break 10:15 – 10:45 AM

Ralph L. Reed and Jan F. Stevens 10:45 – 11:05 AM Lecture Room 326
Oregon State University Smith Hall
Glucosinolate Degradation Products in Fermented Meadowfoam Seed Meal and Their Herbicidal Activities

Sungbeom Lee, Marco Herde, Christiane Gatz, and Dorothea Tholl
Virginia Tech 11:05 – 11:25 AM Lecture Room 326
Smith Hall
Unraveling The Biosynthesis Of Volatiles In Plant Defense: A Single CYTP450 Enzyme Is Responsible For the Conversion of Geranyllinalool to the Insect-induced Homoterpene TMTT in *ARABIDOPSIS THALIANA*

Jim Tokuhisa, Alice Mweetwa, Norma Manrique Constanza, Danielle Hunter, Idit Ginzberg and Richard Veilleux 11:25 – 11:45 AM Lecture Room 326
Virginia Tech Smith Hall
The Steroidal Glycoalkaloids of Solanum Chacoense

Lydia Meador, Patrick T. Walker, Ming-Zhu Shi, and De-Yu Xie
North Carolina State University 11:45 – 12:05 PM Lecture Room 326
Characterization of PAP1-Transgenic Cell Suspension Culture through Growth, Anthocyanins, and Gene Expression

Lunch 12:05 – 1:15 PM Chesapeake Rooms
Student Union

Session VI: Moderator, David Gang, University of Arizona

Neish Speaker
De-Yu Xie
North Carolina State University 1:20 – 1:50 PM Lecture Room 326 Smith Hall

Red cell culture of the PAP1transgene: a functional tool to understanding the regulatory mechanism of MYB75 controlling the biosyntheses of both anthocyanins and proanthocyanidins

Kevin Spelman 1:50 – 2:10 PM Lecture Room 326
Muséum national d'Histoire naturelle Smith Hall

Spilanthol, undeca-2E-ene-8, 10-diynoic acid isobutylamide and extracts from *Spilanthes acmella* demonstrate anti-plasmodial activity

M. S. C. Pedras, S. Hossain, Z. Minic, and Q.-A. Zheng
University of Saskatchewan 2:10 – 2:30 PM Lecture Room 326
Plant Defenses and Pathogen Attack, Tricking Plant Pathogens Smith Hall

Phanikanth V. Turlapati, Laurence B. Davin, Norman G. Lewis,
Washington State University 2:30 – 2:50 PM Lecture Room 326 Smith Hall

Tellimagrandin II Biosynthesis in Tellima grandiflora: C–C coupling STEP

Young Investigators Program 3:00 – 4:30 PM Chesapeake Rooms Student Union

Banquet 6:00 – 7:30 PM Chesapeake Rooms Student Union

Dinner Presentation
Norman Farnsworth 7:30 – 8:30 PM Chesapeake Rooms
University of Illinois at Chicago Student Union

YSONGOCAMRAHP and the Search for Bioactive Chemicals

August 12, 2009 Wednesday

Session VI: Moderator Norman Lewis, Washington State University

Sangeeta Dhaubhadel 9:00 – 9:45 AM Lecture Room 326
Agriculture and Agri-Food Canada Smith Hall
Regulation of Isoflavonoid Synthesis in Soybean Seeds

Mark Bernards 9:45 – 10:30 AM Lecture Room 326
University of Western Ontario Smith Hall
The Ecological Role(s) of Ginsenosides

Lili Zhou, Craig Yencho, DE-Yu Xie 10:30 – 10:50 AM Lecture Room 326
North Carolina State University Smith Hall
HPLC/MS Profiling of Anthocyanin and Carotenes of Sweet potato Genotypes with Variable Flesh Colors

Sung-Jin Kim, Daniel G. Vassão, Laurence B. Davin, Norman G. Lewis;
Washington State University 10:50 – 11:10 AM Lecture Room 326
Formation of Allyl/Propenyl Phenols in Liquid Media: Substrate Versatility of Monolignol Acyltranserases and Allyl/Propenyl Phenol Synthases

Masaomi Yamamura, Shiro Suzuki, Takefumi Hattori, Toshiaki Umezawa
Kyoto University 11:10 – 11:30 AM Lecture Room 326
Smith Hall
The Subunit Composition Of Hinokiresinol Synthase Controls Enantiometirc Composition Of Hinokiresinol Formation

William F. Reynolds
University of Toronto 11:30 – 11:50 AM Lecture Room 326
Smith Hall
The Sensitivity of HMBC Spectra used for Natural Product Structure Determination by NMR can Depend Dramatically on Parameter Choices

RAP Editorial Meetings 12:00 - 2:00 PM Smith Hall 360

INVITED PRESENTATIONS

Key Note Presentation

SPICES AND CULINARY HERBS IN FOLK MEDICINE AND PHYTOMEDICINE

James Duke
The Herbal Garden, Green Pharmacy Garden, 8210 Murphy Road, Fulton, Maryland 20759, JimDuke@comcast.net

In this, the 70th year for the spice Old Bay, now under nearby McCormick Spice Co., Jim Duke in his 80th year, discusses the medicinal potential of the spices in Old Bay. Naturally Jim starts out with Bay itself, Laurus nobilis, since this year 2009, bay, alias laurel, is the Herb Society's' herb of the year. Bay has recently been shown to be rich in the antimalarial phytochemical luteolin. Celery seed, which spared Duke many bout's of gout, is rich in apigenin, cayenne in COX-2-I capsaicin; mustard in chemopreventive isothiocyanates, black pepper in piperine, clove in eugenol, allspice and the antinociceptive myrcene, ginger in gingerols and shogoals, nutmeg in sabinene, cardamom in the anticholinesterase 1,8-cineole and cinnamon in spasmolytic cinnamic acid. Then Duke will go into proven or potential medicinal biologically active phytochemicals in dozens of other Duke believes that, under current technologies, most spices have around 5000 identifiable phytochemicals, almost if not all biologically active and with many pleiomeric activities. And Duke predicts that'll approach 50,000 per spice species. Duke is trying to seek order in this nearly chaotic complexity. His phytochemical database at the USDA, from which he retired in 1995, is still available gratis at http://www.ars-grin.gov/duke. You can find data on 200 spices in this data base. Duke has close to 100 culinary herbs and spices growing in his Green Pharmacy Garden. See http://www.greenpharmacy.com

THE ACTION OF GLUCOSINOLATES/ISOTHIOCYANATES ON *HELICOBACTER PYLORI*

Jed W Fahey, KK Stephenson, KL Wade, P Talalay, M Yamamoto, A Yanaka
Bloomberg School of Public Health-Center for Human Nutrition, Dept. of Pharmacology and Molecular Sciences, Johns Hopkins University, 725 N. Wolfe Street, Baltimore, Maryland 21205 jfahey@jhmi.edu

Three-day-old broccoli sprouts, a widely-available human food, suppress infections with *Helicobacter pylori*, a bacterium that is the cause of the most common infection found in humans worldwide. *Helicobacter* infections are now recognized as the major causes of persistent gastritis and peptic ulcers, and infected people (about half of the world's population) are at 3- to 6-times the risk of stomach cancer as non-infected persons. Although eradication of the infectious agent is the treatment of choice for symptomatic individuals, the need for eradication

(compared to reducing severity of infection), is currently a matter of scientific debate. In addition, resistance to multiple antibiotics has developed in the many *Helicobacter* strains.

We have previously reported upon the potent in-vitro activity of sulforaphane from broccoli sprouts against *H. pylori*[1,2]. We now provide evidence for the beneficial effects of broccoli sprouts in both a mouse model of the *H. pylori* infection and in a clinical intervention on a *Helicobacter*-infected Japanese population[3]. We report studies on mice infected with a human strain of *Helicobacter* and maintained on a high salt diet to aggravate the severity of gastritis. One group of mice received a broccoli sprout puree, rich in a sulforaphane precursor, in the drinking water for 8 weeks, and was compared with a control group receiving no sprouts. The broccoli sprout-treated *Helicobacter*-infected mice showed reduced gastric colonization with *H. pylori*, considerably reduced gastric inflammation and atrophy, lower levels of two inflammatory signaling molecules, and decreased oxidative DNA damage, in comparison to infected control mice.

The human trial involved 48 *Helicobacter*-infected Japanese men and women (age range 23 -73) of whom 25 received fresh broccoli sprouts (70 grams daily for 8 weeks) and 23 consumed an equivalent quantity of fresh alfalfa sprouts during the same period. The severity of *Helicobacter* infection was assessed at the time of enrollment, after 4 and 8 weeks of treatment, and 8 weeks after termination of intervention. The urea breath test (a test routinely used by physicians to assess whether their patients are colonized by *H. pylori* bacteria), and the levels of stool antigens which signal the presence of *Helicobacter* infection, served as biomarkers of the severity of the *Helicobacter* infections. In addition measurement of pepsinogens in the serum served as biomarkers of severity of inflammation of the gastric mucosa. All the values were significantly lowered during the treatment period in subjects consuming broccoli sprouts, but not in the alfalfa (placebo) recipients. These values returned to pretreatment levels 8 weeks after broccoli sprout feeding was discontinued.

These animal and human studies raise the probability that broccoli sprouts can enhance protection of the mucosa lining the stomach against *H. pylori*-induced oxidative stress, improve the course of the infection in human beings, and reduce the risk of stomach cancer. Since stomach cancer is the second most common and the second most deadly cancer worldwide, these findings may have highly important public health implications.

[1] Fahey JW, X Haristoy, PM Dolan, et al. (2002) *Proc. Natl. Acad. Sci. USA* 99: 7610-7615.
[2] Haristoy X, JW Fahey, I Scholtus, & A Lozniewski. (2005) *Planta Medica* 71: 326-330.
[3] Yanaka A, JW Fahey, A Fukumoto, et al. (2009) *Cancer Prevention Research* 2(4): 353-360.

DISCOVERY OF POTENTIAL ANTICANCER AGENTS FROM TROPICAL PLANTS

Douglas Kinghorn, Li Pan, Yulin Ren, Ah-Reum Han, and Ye Deng,
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210-1297, E-mail kinghorn.4@osu.edu.

Natural products from plants and microbes have played an important role in cancer chemotherapy for several decades (1). In a review highlighting oncology drug development over a recent 25-year period it was concluded that a majority of the anticancer drugs approved in North America, Europe, and Japan are either natural products or their derivatives or synthetic molecules based on natural product pharmacophores (2). Currently, our group is collaborating in a multidisciplinary and multi-institutional research project under the "program project" mechanism, in which new natural product anticancer drug leads are obtained from a diverse group of organisms, constituted by tropical plants, aquatic cyanobacteria, and filamentous fungi. Crude extracts are subjected to a panel of cell-based and target-based bioassays, with selected leads then subjected to activity-guided fractionation. The overall organizational plan for this project will be described, as well progress made in the isolation and structural determination of bioactive secondary metabolites from several tropical plants, including *Garcinia lateriflora* Blume (Clusiaceae), *Garcinia mangostana* L. (Clusiaceae), and *Hyptis brevipes* Piot. (Lamiaceae). Through collaborative work with colleagues in The Ohio State Medical Center, it has been found that silvestrol, a 1*H*-cyclopenta[*b*]benzofuran derivative from *Aglaia foveolata* Pannell (Meliaceae), has selective inhibitory effects for B-cell leukemias (4). In an attempt to discover new minor analogs of silvestrol, a large-scale recollection of the stem bark of *A. foveolata* has been investigated phytochemically and biologically. (Funding by grants U19 CA52956 and P01 CA125066 from NCI/NIH is gratefully acknowledged).

References

1. Cragg, G. M.; Kingston, D. G. I.; Newman, D. J., Eds. *Anticancer Agents from Natural Products*; CRC Press/Taylor & Francis: Boca Raton, FL, 2005.
2. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461-477.
3. Kinghorn, A. D.; Carcache-Blanco, E. J.; Chai, H.-B.; Orjala, J.; Farnsworth, N. R.; Soejarto, D. D.; Oberlies, N. H.; Wani, M. C.; Kroll, D. J.; Pearce, C. J.; Swanson, S. M.; Kramer, R. A.; Rose, W. C.; Emanuel, S.; Vite, G. D.; Jarjoura, J.; Cope, F. O. *Pure Appl. Chem.* **2009**, *81*, 1051-1063.
4. Lucas, D. M.; Edwards, R. B.; Lozanski, G.; West, D. A.; Shin, J. D.; Vargo, M. A.; Davis, M. E.; Rozewski, D. M.; Johnson, A. J.; Su, B.-N.; Goettl, V. M.; Lin, T. S.; Lehman, A; Zhang, X.; Jarjoura, D.; Newman, D. J.; Byrd, J. C.; Kinghorn, A. D.; Grever, M. R. *Blood* **2009**, *113*, 4656-4666.

Developing New Botanical Drugs from Medicinal Plants – A FDA Botanical Reviewer's Perspective

Jinhui Dou, Ph.D., Botanical Review Team, Office of Drug Evaluation I (HFD-101), CDER, Food and Drug Administration, Silver Spring, MD

In the United States, botanicals that are known to the consumers as "traditional, alternative & complementary medicines (TACM)" or "herbal medicines, natural health products" are usually fallen into one of the two major categories of FDA regulated products, i.e., dietary supplements and drugs.

There are significant differences between the regulatory approaches for botanical drugs and dietary supplements, and associated requirements on quality, safety and efficacy for their marketing. For dietary supplements, no pre-marketing approval by the FDA is required, however, dietary supplements are not permitted to bear disease claims (only structure/function claims are possible). For drug products, pre-marketing approval by the FDA is required. Besides, drug products are subject to risk/benefit evaluations through non-clinical studies, and more importantly, well-designed clinical trials. Not surprisingly, with tens of thousand of dietary supplement products (many with one or more botanicals), only a handful of botanical drugs are marketed in the U.S.

Since the publication of the Guidance, there has been growing interest in botanical drug development judged by the increasing numbers of botanical INDs and pre-IND consultations, a cumulative total of over 400 and growing. Few of the botanical INDs with phase 1/2 clinical trials have to date advanced into late-phase clinical trials. So far, the Veregen® NDA remains the only one submitted and subsequently approved. Although the reasons for this are no doubt different in different cases, how to produce multiple batches of qualified drug substances (or products) that meet the high quality standards remains as one of the biggest challenges. A discussion of the challenges facing new botanical drug development could shed light on the seemingly low percentage of botanical INDs entering late-phase clinical trials. We, of cause, hope this will change, and that more medical plant derived mixtures (such as active fractions) along with purified compounds will be further developed as new drugs with more success.

ANALYTICAL STRATEGIES FOR ECOLOGICALLY ACTIVE PHYTOCHEMICALS

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Plants produce a wide variety of highly phytotoxic chemicals, some of which have activities comparable to synthetic herbicides. The possibility that plants exert direct chemical, or allelopathic, effects on neighboring plants as well as on soil microflora has attracted considerable research interest. The success of certain invasive plants has been attributed to phytotoxic root exudates. Other work suggests that in conifer forests, monoterpenes released from pine roots and litter inhibit nitrogen mineralization and nitrification. However, there is a general lack of information about the spatial and temporal dynamics of allelochemicals in the soil that is a barrier to testing hypotheses of allelopathic effects. When soils beneath suspected allelopathic plants are analyzed, concentrations are typically low, and this has been cited as evidence that these compounds do not play a significant role in plant-plant interactions. However, static concentrations in the environment reflect the current balance of input vs. output rates for a compound. Because plant roots compete with both microorganisms and other processes that remove allelochemicals from soil solution, flux rates are likely to be a key component of toxicity. Polydimethylsiloxane

(PDMS) sorbents, which are widely used in analytical techniques such as solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE), are being applied to measure the dynamics of thiophenes produced by marigold roots in soil. Various approaches, including PDMS-coated wires and PDMS sheets, have been tested thus far. Results show that the distribution of marigold allelochemicals in the root zone is spatially heterogeneous and dynamic over time. Given the high potency of these thiophenes in bioassays, the amounts recovered can readily be conceived to be biologically active. These techniques appear to be broadly applicable to the analysis of lipophilic root exudates. In addition, SPME has been used to successfully monitor the uptake of soil-applied 1,8-cineole by target plants. Our results indicate that PDMS sorbents are a useful tool for studying ecologically active phytochemicals.

THE EFFECTS OF ALKALOIDS ON THE FEEDING BEHAVIOR AND NEUROPHYSIOLOGY OF INSECTS

Vonnie D.C. Shields, Timothy L. Martin, Katelyn F. Beattie, Nicole S. Arnold, and Kristen P. Smith
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Alkaloids represent one of the largest chemically heterogenous groups of phytochemicals. They occur in 20-30% of higher plants and have approximately 12,000 known chemical structures. They are found most commonly in dicotyledonous angiosperm families, such as Rubiaceae, Apocynaceae, and Solanaceae. Plants containing alkaloids are generally considered to be feeding deterrents for many insects and are important in influencing food selection. Our research is aimed at understanding the role of phytochemicals for insect behavior and physiology. Here, we focus on the effect of alkaloids on the insect larval taste system. Lepidopteran larvae, such as the gypsy moth, possess two pairs of sensory organs on their mouthparts, the lateral and medial styloconic sensilla, which are thought to play an important role in host-plant selection including the detection of phytochemicals, such as alkaloids. These styloconic sensilla each bear five bipolar neurons, four chemosensory and one mechanosensory. They are considered to be the main sensory organs involved in feeding. In one experimental approach, we conducted feeding behavioral bioassays on gypsy moth larvae using a series of alkaloids to determine their potency as feeding deterrents. The results of this study provided innovative insights into the variability of alkaloids as effective feeding deterrents when they were applied to natural versus artificial substrates. When we conducted dose-response experiments, we observed increasing feeding deterrent responses for all the alkaloids tested and found that the alkaloids exhibited different deterrency threshold concentrations. In another series of experiments, we used a single cell electrophysiological tip-recording method to characterize the temporal firing patterns and sensitivities of the receptor cells housed within each styloconic sensillum of gypsy moth larvae in response to stimulation with alkaloids. This method allowed us to evaluate how the gustatory sensory input was encoded as patterns of nerve impulses by receptor cells and sent to processing centers in the brain of the insect.

We found that one receptor cell (i.e., deterrent cell) in both sensilla responded robustly to selected alkaloids and exhibited varying sensitivity, which correlated well with our behavioral results. The results of our study have the potential to suggest deterrent alkaloids as possible novel candidates in designing suitable new strategies for crop protection from insect pests.
This research was supported by NIH grant 1R15DC007609-01 to V.D.S.

DRUG DISCOVERY OF NATURAL PRODUCTS FOR PHARMACOGNOSY

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Natural products offer a vast and virtually unlimited source of new agents for both the pharmaceutical and agrochemical industries. The National Center for Natural Products Research (NCNPR) was created to bring together an alliance of academia, government, and the pharmaceutical and agrochemical industries to integrate research, development, and commercialization of potentially useful natural products.

Development in science and technology has impacted every aspect of our lives. The most precious thing among them is health. We all agree that everyone should have access to health coverage and access to medicine but in reality its becoming difficult even in well developed countries due to high cost of it. The model of modern medicine is not addressing the current need and the need of the future. Eighty percent of the world population still relies on traditional medicine.

The same time natural products are becoming more popular as people are becoming more health conscious. Natural products can be developed as a drug or as a herbal medicine. The main issue for making herbal preparation as well respected medicine is standardization. Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question.
Recent developments in natural products chemistry at NCNPR related to drug discovery and herbal products will be presented.

A PROTEIN WITH STRONG ANTI-INFLUENZA ACTIVITY FROM THE ANTARCTIC RED MARINE ALGA *GIGARTINA SKOTTSBERGII*

J. Alan Maschek[1], Cindy Bucher[2], Alberto van Olphen[2] and Bill J Baker.[1] [1]University of South Florida, Department of Chemistry, [2]University of South Florida, Department of Global Health, 4202 E. Fowler Ave, Tampa, FL, 33620, bjbaker@cas.usf.edu

With an estimated 3 to 5 million infections and as many as 500,000 deaths from the complications of influenza infections each year, there lies a critical need to identify novel drug classes and structures which can be exploited for antiviral development. A bioassay-guided fractionation of extracts from the red marine algae *Gigartina skottsbergii*, collected near Anvers Island, Antarctica, significantly inhibited the reproduction of influenza virus A/Wyoming/3/2003 (H3N2) in MDCK cells *in vitro* with an IC_{50} value of 4 µg/mL. The virus-inhibitory effect was selective, dose-dependent, strain-specific and the virus induced cytopathogenic effect (CPE) was reduced at non-toxic concentrations of the extract. SDS-Gel electrophoresis and sequencing of the active fraction reveals homology with lectins. Insight into the mechanism of action via hemagglutination and plaque assay suggests cell protection by interference of viral docking. Our research suggests that activity from current over-the-counter anti-influenza vitamin supplements from the same genus and species does not arise from the previously reported antiviral sulfated polysaccharides.

A LITTLE BIOLOGY GOES A LONG WAY: EVALUATION OF EXTRACTION METHODS FOR GALLIC ACID, CATECHINS AND XANTHINES IN GREEN TEA

Michael C. Tims and Lane C. Sander
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Green tea (*Camellia sinensis*), a popular beverage enjoyed worldwide, has been reported to contain compounds used in treatment of cancer, genital warts, cardiovascular disease, and as an antimicrobial. Accurate measurement and detection of these compounds is necessary for assuring good manufacturing practices and in the conduct of clinical trials. As part of a collaborative effort between the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH) Office of Dietary Supplements analytical methods have been developed for the determination of catechins, gallic acid, theanine and methyl xanthines in a suite of green tea containing standard reference materials (SRMs).

The metabolic pool of catechins in green tea leaf tissue can be altered by both biotic factors and tea leaf processing. Traditional green tea leaf extraction methods underestimated 'true' analyte levels in green tea leaves, particularly levels of epigallocatechin gallate (EGCG). Understanding the biology and chemistry of catechins, plant primary cell walls and the antimicrobial properties of catechin galloyl esters helped guide analytical method development. Enzyme assisted extraction using of 0.1 % EDTA combined with a secondary pressurized fluid extraction increased the recovery of catechins and limited interference of

biopolymer fragments to optimize extraction efficiency. This talk describes efforts to develop quantitative extraction procedures for use in the certification of green tea containing SRMs currently under development.

YSONGOCAMRAHP AND THE SEARCH FOR BIOACTIVE CHEMICALS

Norman R. Farnsworth
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An overview of the taxonomic distribution of phytochemicals and classes of biological activity for plant species will be presented. A cornucopia of data, ranging from clinical trials to *in vitro* analysis of botanical extracts and pure compounds, to reports on the ethnomedical use of plants, will be considered, compared and contrasted. Tidbits and anecdotes, designed to edify, aid digestion, and keep the audience awake, will be offered.

REGULATION OF ISOFLAVONOID SYNTHESIS IN SOYBEAN SEEDS

Sangeeta Dhaubhadel
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Isoflavonoids are a diverse group of secondary metabolites that accumulate in soybean seeds during development. They play important roles in the interaction between plants and the environment. Several clinical studies have suggested a role for isoflavonoids in human health and nutrition. The amount of isoflavonoids present in soybean seeds is influenced by both genetic and environmental factors that are not fully understood. Our studies suggest that total isoflavonoid accumulation in soybean seed is a result of *de novo* synthesis within the seed as well as transport from maternal tissues. We have conducted a global gene expression analysis of developing soybean seeds in two cultivars that differ in the level of isoflavonoid accumulation. The results established that the differential expression of *chalcone synthase* (*CHS*)*7* and *CHS8* genes determines the differences in the levels of isoflavonoid accumulation in soybean seed. A search for factor (s) that regulate the expression of the key isoflavonoid biosynthetic genes has identified a MYB family of

transcription factor that recognizes and binds with specific sequence within the *CHS8* promoter and regulates the *CHS8* gene expression. The direct evidence of the involvement of the MYB factor in isoflavonoid biosynthesis was confirmed by RNAi silencing.

THE ECOLOGICAL ROLE(S) OF GINSENOSIDES

Mark A. Bernards[1], Dimitre Ivanov[1], Andreea Neculai[1], Lina Yousef[2] and Robert Nicol[3]
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Ginsenosides are triterpenoid saponins produced by *Panax* spp (ginseng), and are purported to possess medicinal properties. However, we wanted to know why ginseng makes these compounds, since they accumulate up to 6-7% of the dry weight of roots. In our initial experiments we established that, like other saponins, ginsenosides are fungitoxic (albeit only mildly) to some soil borne microorganisms *in vitro*; however, we also demonstrated that the growth of other microorganisms (especially aggressive pathogens of ginseng plants) was enhanced in the presence of ginsenosides. Further, we noted that the profile of ginsenosides recovered from the medium of pathogens such as *Pythium irregulare* was significantly altered. That is, when *P. irregulare* was cultured in the presence of a purified (>90%) ginsenoside mixture, nearly all of the 20(*S*)-protopanaxadiol ginsenosides (Rb_1, Rb_2, Rc, Rd, and to a limited extent G-XVII) were metabolized into the minor ginsenoside F_2, at least half of which appeared to be internalized by the organism. No metabolism of the 20(*S*)-protopanaxatriol ginsenosides (Rg_1 and Re) was evident. The metabolism of ginsenosides was dependent on extracellular glycosidases secreted into the culture medium by the pathogen. To further explore the apparent selective metabolism of 20(*S*)-protopanaxadiol ginsenosides by extracellular enzymes of *P. irregulare*, we purified and partially sequenced three glycosidase enzymes, and demonstrated them to be acidic proteins (pI of 4.5-5.0), consisting of an apparent high molecular weight (~160 kDa) homodimer of 78 kDa subunits, with β(1→6) activity, and two monomeric enzymes of 61 and 57 kDa, with β(1→2) activity. Importantly, the appearance of these specific glycosidases in the culture medium of *P. irregulare* is dependent on the presence of ginsenosides in the culture medium, suggesting that their expression is triggered by the presence of these compounds. We speculate that the role of these extracellular glycosidases is likely to help *Pythium* find its host, and/or obtain nutrients/growth factors from its environment.

NEISH SPEAKERS

SALVAGE OF FOLATE CATABOLITES IN PLANTS: BIOCHEMICAL CHARACTERIZATION OF p-AMINOBENZOYLGLUTAMATE HYDROLASE

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Folates are essential cofactors for one-carbon transfer reactions in most organisms, but are made only by plants and microbes. Folates are comprised of a pterin, *p*-aminobenzoate (*p*ABA), and glutamate moieties. Although the enzymatic steps governing folate biosynthesis are well described in plants, very little is known about folate catabolism and salvage. Folates are quite susceptible to oxidative breakdown, yielding a pterin and *p*-aminobenzoylglutamate (*p*ABAGlu) or its polyglutamates.

In plants, folate degradation is on the order of 10% per day. Salvage of *p*ABAGlu is suggested by the following: (i) *p*ABAGlu does accumulate relative to folate pools; (ii) *Arabidopsis* and tomato tissues readily converted supplied *p*ABAGlu to the folate synthesis precursor, *p*ABA, and glutamate; and (iii) *in vitro* studies confirmed the presence of *p*ABAGlu hydrolase (PGH) activity in plants. Biochemical characterization of PGH activity suggests the presence of several isoforms in both *Arabidopsis* and pea. A partially purified *Arabidopsis* root PGH hydrolyzes *p*ABAGlu, as well as folate. This activity was strongly inhibited by a metal chelator and stimulated by Mn^{2+}, pointing to a metalloenzyme. The *Arabidopsis* genome was searched for proteins similar to *Pseudomonas* carboxypeptidase G, which contains zinc and is the only enzyme yet confirmed to attack *p*ABAGlu. The sole significant matches were auxin conjugate hydrolase family members and the At4g17830 protein. None was found to have significant PGH activity, suggesting that this activity resides in hitherto unrecognized enzymes. The finding that *Arabidopsis* has folate-hydrolyzing activity points to an enzymatic component of folate degradation in plants.

COMT (CAFFEIC ACID *O*-METHYL TRANSFERASE) MUTATION AND EFFECTS ON BOTH LIGNIN PRIMARY STRUCTURE AND DECONSTRUCTION

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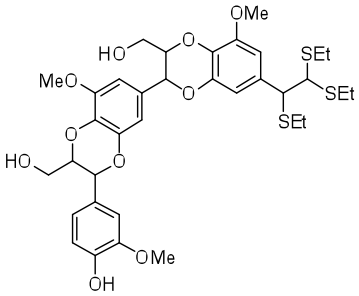
"Caffeic acid *O*-methyl transferase" (COMT) was originally viewed as being a bispecific OMT based on *in vitro* assays and transgenic manipulations, i.e. whereby it was thought that it was able to *O*-methylate caffeic acid and 5-hydroxyferulic acid moieties *in vivo* in the monolignol pathway.

Atanassova *et al*., 1995, however, demonstrated unambiguously that "COMT" only catalyzes the regiospecific methylation of 5-hydroxy-G (5-OH-G) units (G = guaiacyl) substrates in angiosperms, this resulting in formation of the so-called S (syringyl) lignin. These studies also established, however, that down-regulation of "COMT" in transgenic tobacco lines resulted in near abolition of the syringyl (S) component in lignins, whereas

there was apparently little to no alteration in the G levels of the lignin formed, i.e. suggesting that "COMT" was a 5-hydroxyguaiacyl *O*-methyl transferase. We now report that the lignin primary structure in *Arabidopsis* COMT mutated lines has benzodioxane substructures in its lignin whose overall 8-*O*-4′ inter-unit linkage frequency is apparently being conserved relative to wild type. These subunits are, however, readily cleavable as 5OH-G/G and 5OH-G/5OH-G/G moieties in the lignin. Specifically, plant cell wall lignin deconstruction, and identification of the released subunits by NMR spectroscopic and wet chemical analyses, together with confirmation by chemical syntheses, have provided further evidence for lignin macromolecular assembly being conserved i.e. in a highly programmed (template guided) manner. In this case, formation of 5-OH-G units results in these moieties being 8-*O*-4′ linked to the lignin primary structure as depicted



Cleavable subunits in COMT-derived lignin

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RED CELL CULTURE OF THE PAP1TRANSGENE: A FUNCTIONAL TOOL TO UNDERSTANDING THE REGULATORY MECHANISM OF MYB75 CONTROLLING THE BIOSYNTHESES OF BOTH ANTHOCYANINS AND PROANTHOCYANIDINS

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PAP1 is a plant MYB R2R3 transcription factor. Arabidopsis *pap1-D* (*production of anthocyanin pigment 1-Dominant*) is a dominant mutant generated by T-DNA activation tagging (Borevitz *et al.*, 2000). The insertion of 4 x 35S enhancer sequences adjacent to the start of the PAP1 gene results in the overexpression of this transcription factor, which leads to the enhanced accumulation of anthocyanins in most of the organs. Several previous reports showed that the overexpression of PAP1/MYB75 could enhance or activate several pathway genes of both the anthocyanins and proanthocyanidins. A recent ecological study showed that the anthocyanin formation in Arabidopsis could be independent of the transcription of the PAP1. Calli were induced from leaves of *pap1-D* mutants and PAP1-transgenic tobacco in our laboratory. Induction of cell differentiation led to obtainment of several different PAP1-transgenic cell lines of tobacco, including red and white cell lines,

which transcribed a similar level of the PAP1 transgene, but the white cells either did not produce anthocyanins or produced a only trace level of anthocyanins. In this report, I will present and discuss the use of the red cell culture as a functional tool for a systems study of regulatory mechanism of MYB75.

CONTRIBUTED PRESENTATIONS

THE BIOCHEMICAL EVOLUTION OF PHYTOMEDICINES

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Structurally complex bio-medicinal molecules in plants are primarily the result of millions of years of co-evolutionary interactions between plants and herbivores. These herbivores or predators, include insects, herbivorous animals, fungi, bacteria and viruses, with a latitudinal gradient in bioactivity evident from the tropics (more complex and bioactive molecules versus plants of the cold temperate zones). It is therefore, not surprising, that some natural poisons are of medicinal value, since they inhibit primary processes, enzymes, kinases, proteases, form protein adducts and induce apoptosis in insects and in human cancer cells. In this presentation, the evolution, chemistry and antitumor activities of novel bark spiro-lignans from tropical plants are presented, followed by a brief discussion on how plant medicinal use was acquired by the early apes (zoopharmacognosy) and learned by the early hominids.

ISOLATION AND CHARACTERIZATION OF THE *KALANCHOE DAIGREMONTIANA* CYCLASES FORMING THE CUTICULAR TRITERPENOIDS GLUTINOL AND FRIEDELIN

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Pentacyclic triterpenoids are a large group of secondary metabolites found in many different plant species, either as glycoside conjugates or as aglycones. The first committed step in triterpenoid biosynthesis is the cyclization of 2,3-oxidosqualene into various isomeric products $C_{30}H_{50}O$. The transformation proceeds via epoxide protonation, multiple ring formation reactions, 1,2-methyl and 1,2-hydride shifts and a final deprotonation step, all catalyzed by a single enzyme. Different multifunctional triterpenol synthases and/or combinations of several product-specific enzymes account for the diversity of triterpenoids found in each plant species. One major difference between all these enzymes is how many 1,2-shifts they facilitate before releasing the final products. All the triterpenoid synthases described to date catalyze reactions involving only a few 1,2-shifts, and relatively little is

known about the rearrangement mechanisms beyond that point. Therefore, the goal of this project was to identify and characterize triterpenoid synthases catalyzing a maximum number of 1,2-shifts leading to the formation of glutinol and friedelin.

Two novel triterpenoid synthase genes were isolated from *Kalanchoe daigremontiana* and heterologous expression in yeast, followed by GC-FID and GC-MS analysis, showed that they code for a friedelin synthase and a glutinol synthase. Sequence comparisons between both enzymes, together with three-dimensional structure modeling, were used to predict amino acids involved in determining product specificity. To test these predictions, chimeragenesis and site-directed mutagenesis experiments were carried out. The product profiles of the altered enzymes were significantly changed, most notably in the percentages of glutinol and friedelin. The implications of these results for the mechanisms of triterpenoid cyclization will be discussed.

Our findings also have implications for the biological functions of plant triterpenoids. Friedelin and glutinol accumulate to high concentrations in the cuticular wax of *K. daigremontiana*, where they likely play an important role in protecting the plant against biotic and/or abiotic stress. As a result, understanding the genetics and biochemistry of triterpenoids will also provide us further insight into their physiological and ecological roles.

BIOACTIVE PHYTOCHEMICALS IN MEDICAL FOODS FOR PHYTOTHERAPY

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Medical foods are finding their way on to pharmacy shelves as prescription only items. The area of food as medicine is amorphous and the term medical food conveys this unique confusion. Medical foods are in reality not foods. Medical foods are usually some form of dietary supplement composed of bioactive phytochemicals, singular or composite, of GRAS status in a non-food matrix. The objective of this paper is to explore the major issues of bioactive phytochemicals in medical foods that are used for phytotherapy in the United States. These major issues are: regulation, safety, efficacy, synergy, and pharmacovigilance. The regulatory issue is problematic for medical and pharmacy practice due to the fact that the FDA does not require safety or efficacy evaluation for medical foods. The FDA classifies medical foods not as drugs, but as foods. Type 2 medical foods have prescription only status. There are no evaluation standards for medical foods in the U.S. and this fact makes the issue extremely difficult for healthcare professionals. This is a quagmire. The issues of safety and efficacy clearly suffer due to this regulatory quagmire. Because of this singular regulatory environment for medical foods some companies use this loop hole to market products that after review by experts have been found to be of unproven efficacy.

Flavocoxid is an example. Complicating the clinical picture is the fact that some phytochemicals with bioactivity can be sourced from non-plant natural sources such as Co-enzymeQ-10. It is worth mentioning that Co-Q-10 can exist as a medical food (ubiquinol) or a dietary supplement (ubiquinone). Phytotherapy has vast potential in the emerging area of integrative therapeutics. This promising area is due in part to the unique aspect of bioactive phytochemicals in multi-constituent products commonly exhibiting synergy. This unique area of phytochemical synergy in multi-constituent natural products is informed by the pleiomorphic expression of physiological and pharmacological effects of individual phytochemicals and phyto-combinations in food and non-food matricies. The current issue of pharmacovigilance is of great importance to modern U.S. public health. This area is certainly topical to phytotherapy from the standpoint of phytochemical-drug interactions. Black pepper extract is a constitiuent in at least one medical food. This product contains a specific formulation of (98%) piperine at 5mgs. Black pepper extract has the potential for the inhibition of several P-450(CYP) isoforms and p-glycoprotien substrates. Levels of evidence evaluation shows that there are many possible clinically important drug interactions with piperine, and at least one probable interaction of piperine with lithium. This area of pharmacovigilance needs urgent attention in medical and pharmacy education and practice. The regulatory issues that currently plague medical foods will resolve as bioactive phytochemicals undergo conclusive clinical trials for safety and efficacy. Bioactive phytochemicals (both those with-in the foods matrix and those with-in a non-food matrix) will become first-line treatment options in the emerging paradigm of integrative phyto-pharmacotherapy as U.S. medical and pharmacy practice evolve. The FDA is hard at work trying to find ways to deal with the quagmire of the regulatory status of functional foods, dietary supplements, and medical foods. It is a dynamic and exciting time to be active in bioactive phytochemicals.

CHARACTERIZATION OF PAP1-TRANSGENIC CELL SUSPENSION CULTURE THROUGH GROWTH, ANTHOCYANINS, AND GENE EXPRESSION

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Anthocyanins are plant flavonoid pigments providing pink/red/blue/purple coloration in most plants. They attract both pollinators and seed dispersers. In addition, their antioxidative activity protects plants from radiation-induced damage on cells. Furthermore, their presence in food provides strong antioxidative uses benefiting human health. PAP1 is one of the R2R3-MYB transcription factors directly regulating the biosynthesis of anthocyanins. We have established PAP1-transgenic callus and suspension cell cultures of tobacco. In this study, we characterize the growth of cells, anthocyanin formation, and gene expression in suspension-cultured transgenic red cells (6R) of PAP1 during a 25-day culture period. Transgenic white cells (6W) of PAP1 and wild-type cells (P3) were used as controls. Samples were collected from six time points at days 0, 5, 10, 15, 20, and 25. Growth curves for the change in fresh weight were measured for these suspension culture cells. The biomass increase of the 6R cells followed a sigmoid-like trend.

The biomass increase of the P3 cells followed a logarithmic trend. The fresh weight increase of the P3 cells was faster than that of the 6R cells. A dynamic trend of anthocyanin levels were observed in the 6R cells. The P3 cells did not produce anthocyanins. In addition, we will present and discuss the anthocyanin structures and profiles and the expression levels of the PAP1 transgene and its targeted pathway genes (e.g. PAL, CHS, DFR, and ANS). This research is funded USDA-NRI (proposal number 2006-1334).

XANTHOHUMOL AND RELATED PRENYLATED FLAVONOIDS FROM HOPS INHIBIT PRODUCTION OF INFLAMMATORY MEDIATORS IN ACTIVATED MONOCYTES AND MACROPHAGES

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Xanthohumol is a prenylated chalcone-type flavonoid found in hops (*Humulus lupulus*), beer, and in several dietary supplements currently on the market. Our objective of this study was to determine the anti-inflammatory activities of xanthohumol, isoxanthohumol, and 17 related flavonoids as well as to determine structure-activity relationships. The isoflavone, genistein, and the flavonol, quercetin, were also included in the study. The anti-inflammatory activities of the 21 flavonoids were measured by their ability to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse macrophage RAW 264.7 and to inhibit LPS-induced cytokine production in human monocytic THP-1 cells. 8-Geranylnaringenin and xanthohumols B and C were most active as inhibitors of LPS-induced NO production in RAW 264.7 cells at 20 μM. In THP-1 cells, 8-geranylnaringenin was the most active inhibitor of LPS-induced production of MCP-1 and IL-6 followed by 6-geranylnaringenin and isoxanthohumol. Addition of prenyl groups to naringenin and chalconaringenin as well as substitution of prenyl groups with geranyl groups showed a profound increase of inhibitory effects on MCP-1 and IL-6 production in THP-1 cells, which cannot be explained by the resulting increase in lipophilicity. The type of flavonoid (chalcone, dihydrochalcone, flavanone, isoflavone, or flavonol) appeared to have a minor impact on inhibitory activity.

IDENTIFICATION, BIOCHEMICAL CHARACTERIZATION, AND STRUCTURE FUNCTION ANALYSIS OF A FLAVONOL 3-O-GLUCOSYLTRANSFERASE FROM *CITRUS PARADISI*

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Glucosylation is a predominant flavonoid modification reaction affecting the solubility, stability, and subsequent bioavailability of these compounds. In citrus, flavonoid glycosides affect taste characteristics making the associated glucosyltransferases particularly interesting targets for biotechnology applications. In this work, a grapefruit (C*itrus paradisi* var. Duncan) glucosyltransferase gene (GQ141630) was identified, cloned, and introduced into the pET recombinant protein expression system utilizing primers designed against a predicted flavonol/anthocyanidin glucosyltransferase gene (AY519364) from blood orange (*Citrus sinensis* var. tarocco). The encoded *Citrus paradisi* protein is 51.2 kDa with a predicted pI of 6.27 and is 96% identical to the *Citrus sinensis* homologue. A number of compounds from various flavonoid subclasses were tested, and the enzyme glucosylated only the flavonol aglycones quercetin (K_m^{app}=67 µM; V_{max}=20.45 pKat/µg), kaempferol (K_m^{app}= 12 µM; V_{max}= 11.63 pKat/µg), and myricetin (K_m^{app}= 33 µM; V_{max}= 12.21 pKat/µg) and did not glucosylate the anthocyanidin aglycone, cyanidin. Glucosylation occurred at the 3 hydroxyl position as confirmed by HPLC and TLC analyses with certified reference compounds. The optimum pH was 7.5 with a pronounced buffer effect noted for reactions performed in Tris-HCl buffer. The enzyme was inhibited by Cu^{2+}, Fe^{2+}, and Zn^{2+} as well as UDP (K_i^{app} = 69.5 µM), which is a product of the reaction. Treatment of the enzyme with a variety of amino acid modifying compounds suggests that cysteine, histidine, arginine, tryptophan, and tyrosine residues are important for activity. The thorough characterization of this *Citrus paradisi* flavonol 3-O-glucosyltransferase adds to the growing base of glucosyltransferase knowledge, and will be used for continued investigations into structure function relationships.

PLANT DEFENSES AND PATHOGEN ATTACK, TRICKING PLANT PATHOGENS

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The “arms race” between plants and their attackers whether microbes or pests, is an outcome of co-evolution that continues to cause enormous crop losses. To devise sustainable methods to prevent and deter plant pathogens, the molecular interaction between crucifers and their pathogenic fungi is under intense investigation. Cruciferous plants (e.g. canola, mustard, cauliflower, cabbage, turnip, etc.) produce complex blends of metabolites with diverse ecological roles such as self-protection against microbial pathogens, pests and other sorts of stress, while their fungal pathogens produce phytotoxic metabolites that facilitate plant invasion. Phytoalexins (induced) and phytoanticipins (constitutive) of crucifers have a very wide range of structures and biological activities. From our results, it is apparent that many of these natural products involved in crucifer defense reactions (both constitutive and induced metabolites) are detoxified by their fungal pathogens. Our screening results using synthetic and natural compound libraries have shown that these fungal detoxifications can be inhibited. Taking as lead structures a few plant natural defenses, a new generation of inhibitors of fungal enzymes were designed

and shown to be specific against particular pathogens. The most recent metabolic studies and challenges of this strategy to treat plant fungal diseases will be presented.

TELLIMAGRANDIN II BIOSYNTHESIS IN *TELLIMA GRANDIFLORA*: C–C COUPLING STEP

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Ellagitannins are an important class of hydrolysable tannins distributed across a wide range of plant species, and to date at least 500 ellagitannins have been structurally characterized. Besides their intrinsic antioxidant properties, some are bioactive (including those with antitumour, antiviral and anticancer activities). However, the biosynthetic pathway leading to formation of this structurally diverse group is poorly understood, at least in terms of monomeric radical-radical coupling. In this context, some of the major biosynthetic routes in ellagitannins are considered to result from oxidative C–C coupling of galloyl groups and oxidative C–O coupling leading to their oligomerization. Yet, very few studies have investigated the biochemical aspects of these coupling mechanisms. In *Tellima grandiflora*, putative laccases were initially reported as involved in oxidative C–C intramolecular coupling leading to formation of tellimagrandin II (monomer) and C–O intramolecular coupling to form cornusiin E (dimer). In our studies, however, no evidence to support laccase involvement in either biochemical pathway could be made. The present study was thus aimed to understand clearly the enzyme(s) involved in the oxidative coupling transformation(s) leading to tellimagrandin II. From our ongoing studies, an enzyme involved in C–C coupling leading to formation of tellimagrandin II from 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose as a substrate was detected and localized to isolated chloroplasts, with the latter being purified by percoll gradients. Similar results were also obtained using purified leaf protoplasts for *in vitro* reactions. Since no plant laccases have yet been reported as localized to chloroplasts, the existence of another class of oxidases is most likely. We propose polyphenol oxidases as the preferred candidates because of their localization to chloroplasts. Efforts are underway to obtain pure chloroplasts in bulk so that the enzyme(s) can be isolated to homogeneity. The results from the present study partially address the previous enigma of enzymatic oxidative transformations to this broad class of hydrolysable tannins. These findings herein thus place the coupling enzyme in the same subcellular compartments as for the substrate and products.

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RED CALLUS CULTURE OF ARABIDOPSIS AS A TOOL FOR ANALYZING THE REGULATION MECHANSIM CONTROLLING THE FUNCTION OF PAP1 TRANSCRIPTION FACTOR

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PAP1 is a plant MYB R2R3 transcription factor. Arabidopsis *pap1-D* (*production of anthocyanin pigment 1-Dominant*) is a dominant mutant generated by T-DNA activation tagging (Borevitz *et al.*, 2000). The insertion of 4 x 35S enhancer sequences adjacent to the start of the PAP1 gene results in the overexpression of this transcription factor, which leads to the enhanced accumulation of anthocyanins in most of the organs. Calli were induced using *pap1-D* leaves as explants. Several anthocyanin-producing callus lines were obtained after multiple times of subculture and selection. As a control, wild-type (WT) callus culture was also established in the same condition. Seven anthocyanins in *pap1* callus were identified by liquid chromatography mass spectrometry (HPLC-MS) analysis. Hydrolysis and LC-MS analysis showed that cyanidin formed the predominant core structure of these anthocyanins. RT-PCR result confirmed that the accumulation of anthocyanins in the red callus were result from the overexpression of the PAP1 gene. The expression levels of the anthocyanin pathway genes, especially late pathway genes (eg. *DFR* and *ANS*), were dramatically increased in the red calli in comparison with the WT calli.

This red calli culture and the biosynthesis of anthocyanins provided a useful tool to understand the regulation mechanism of PAP1 in Arabidopsis cells and the mechanisms of how other factors control the activity of this transcription factor. We investigated the effects of different nitrogen sources and their concentrations on the biosynthesis of anthocyanins. We tested three combinations of NH_4NO_3 and KNO_3 concentrations in the MS medium: 20 mM NH_4NO_3 and 18.8 mM KNO_3, 10 mM NH_4NO_3 and 9.4 mM KNO_3, 0 NH_4NO_3 and 9.4 mM KNO_3. Higher concentration of NH_4NO_3 and KNO_3 used in the MS medium inhibited the biosynthesis of anthocyanins and changed the profiles of anthocyanins. Semi-quantitative RT-PCR analysis showed that higher concentrations of these two nitrogen nutrients down regulated the transcriptional levels of *PAP1* and 4 analyzed pathway genes (*PAL, CHS, DFR*, and *ANS*). The effects of the PAP1 overexpression on global transcriptome will be discussed in this presentation.

SPILANTHOL, UNDECA-2E-ENE-8, 10-DIYNOIC ACID ISOBUTYLAMIDE AND EXTRACTS FROM *SPILANTHES ACMELLA* DEMONSTRATE ANTI-PLASMODIAL ACTIVITY

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Galenic extracts from *Spilanthes* spp. are actively used to treat malaria in Africa and India. Yet there is thus far no data on active constituents or most effective extraction methods. Spilanthol (deca-2E,6Z,8E-trienoic acid isobutylamide) the predominant alkylamide in *Spilanthes* spp., and undeca-2E-ene-8,10-diynoic acid isobutylamide, a lesser occurring alkylamide, were found to have IC50s at 15 µg/mL and 40 µg/mL, respectively, on *Plasmodium falciparum* strain PFB and 6 µg/mL and 18 µg/mL on the chloroquine resistant *P. falciparum* K1 strain. Further investigations showed that spilanthol and the water extract of *S. acmella* reduced the parasitemia 53% and 59% in mice infected with *P. yoelii yoelli* 12XNL at 5 µg/mL and 50 µg/mL, respectively. The 95% ethanol extract of *S. acmella* was,

unexpectedly, less effective (36% reduction in parasitemia) at 50 µg/mL. This provides the first evidence supporting *S. acmella* against malaria and provides the first evidence demonstrating active constituents in *S. acmella* against *P. falciparum.*

GLUCOSINOLATE DEGRADATION PRODUCTS IN FERMENTED MEADOWFOAM SEED MEAL AND THEIR HERBICIDAL ACTIVITIES

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Meadowfoam (*Limnanthes alba*) is an oilseed crop in western Oregon. The seed meal contains 2-4% of the glucosinolate, glucolimnanthin, and minor amounts of other glucosinolates. We have developed a method for the conversion of seed meal glucosinolates into its degradation products by fermenting enzyme-inactive seed meal with small quantities of enzyme-active meadowfoam seeds. Glucolimnanthin was converted into the corresponding isothiocyanate, nitrile, and thioamide. The thioamide degradation product, 2-(3-methoxyphenyl)-ethanethioamide, was identified by isolation and HPLC-UV and LC-MS/MS comparison with a synthetic standard. This chemical has not previously been reported as a naturally occurring compound. We were able to increase the yield of 3-methoxybenzyl cyanide (nitrile) and the thioamide degradation product by co-incubation of seed meal with enzyme-active seeds and $FeSO_4$ (10 mM), at the expense of the corresponding isothiocyanate. In collaboration with Dr. Machado (Columbia Basin Agricultural Research Center, Pendleton, Oregon, OSU), the herbicidal activity of glucolimnanthin and the isothiocyanate, nitrile, acetamide, and thioamide degradation products was determined in a coleoptile emergence assay using downy brome (*Bromus tectorum*) seeds planted on a layer of soil. The order of herbicidal potency was found to be: glucolimnanthin (least potent) < isothiocyanate < acetamide < nitrile < thioamide (most potent). Fermentation of meadowfoam seed meal by treatment with active *L. alba* seeds significantly increased the herbicidal potency compared to no treatment or sham treatment. The herbicidal activity was further enhanced by treatment of seed meal in the presence of $FeSO_4$. The herbicidal potency of the treated (fermented) meals correlated well with the content of the isothiocyanate, nitrile and thioamide degradation products.
Acknowledgments – *This research is funded by USDA grant CSREES 2005-34407-15670 and by Natural Plant Products, Inc., Salem, Oregon*.

UNRAVELING THE BIOSYNTHESIS OF VOLATILES IN PLANT DEFENSE: A SINGLE CYTP450 ENZYME IS RESPONSIBLE FOR THE CONVERSION OF GERANYLLINALOOL TO THE INSECT-INDUCED HOMOTERPENE TMTT in *ARABIDOPSIS THALIANA*

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Volatile secondary metabolites such as homoterpenes released from vegetative plant tissues have significant functions in plant defense by directly warding-off herbivorous insects or attracting natural enemies of herbivores. This intriguing defense system has drawn increasing attention to the metabolic engineering of volatiles as a tool in developing alternative pest controls. The C_{16}-homoterpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) is emitted from aerial parts of many plants including crops such as maize, lima bean, tomato and alfalfa as well as the model plant *Arabidopsis thaliana.* TMTT emission is induced upon insect and mite attack and functions in attracting parasites or predators of these pests. Besides insect feeding, emission of TMTT can also be induced by fungal elicitors and bacterial pathogen infection.

We are interested in identifying the key enzymatic steps in the TMTT metabolic pathway. Here we demonstrate for the first time that CYP82G1 (At3g25180), a CytP450 enzyme of the *Arabidopsis* CYP82 family, is responsible for the single-step conversion of the C_{20}-precursor geranyllinalool to TMTT. We describe that expression of the *CYP82G1* gene is coordinated with that of geranyllinalool synthase in *Arabidopsis* leaves upon treatment with the fungal elicitor alamethicin, particularly in a COI-1 dependent manner. TMTT emission is absent in *CYP82G1* loss-of-function mutants under elicitor-induced conditions but can be restored by complementation with the *CYP82G1* gene expressed under control of the constitutive CaMV35S promoter. Notably, *in vitro* enzyme assays with recombinant CYP82G1 and *in vivo* substrate-feeding assays in yeast cells over-expressing *CYP82G1* reveal a broader substrate specificity of the enzyme by converting not only geranyllinalool but also its C_{15}-analog nerolidol to the respective C_{11}-homoterpene DMNT. Histochemical *CYP82G1* promoter-GUS assays confirm inducible expression of this gene in leaves upon insect feeding and show that constitutive expression is limited to stems and inflorescences.

THE STEROIDAL GLYCOALKALOIDS OF SOLANUM CHACOENSE

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Department of Horticulture, Virginia Tech ; [2]Agricultural Research Organization, Volcani Center, Israel; email: tokuhisa@vt.edu

Steroidal glycoalkaloids are a class of plant natural products that are produced most abundantly by species of the Solanoideae subfamily of the Solanaceae. They are glycosides of nitrogen-containing steroidal derivatives. These compounds are recognized as plant defense compounds that are effective against herbivore and fungal attack. The Solanoideae includes the two most important vegetable crops produced world-wide--potato and tomato. The wild progenitors of cultivated *Solanum* can contain in excess of 20 mg of SGAs

per 100 grams fresh weight, which are levels that are considered unsafe for human consumption because of the toxic cellular properties of SGAs as anticholinesterases and membrane disruptors. Prehistoric breeding practices initiated, and contemporary breeding programs have maintained levels of SGAs in new cultivars that are low enough for safe consumption of potatoes but high enough to contribute to the familiar and safe organoleptic properties of unripe green tomatoes and potato skins. Despite the importance of SGAs as natural pesticides and as potential human toxins, we have a limited understanding of how they are made.

To provide a molecular genetic basis for future *Solanum* breeding, we are using biochemical, molecular, and genetic approaches to identify the enzymes comprising the novel biosynthetic pathway of steroidal glycoalkaloids with a particular interest in the enzymes associated with steroidal alkaloid formation. We have isolated and characterized a gene coding for squalene synthase from a wild potato species. We are developing a high throughput protocol to extract and purify SGAs in cultivated and wild *Solanum* and are optimizing separation technologies for HPLC and LC-MS to quantify and identify SGAs. In addition, we are developing methods for transient gene expression in *Solanum* to screen for genes involved in SGA biosynthesis.

To understand SGA formation in *S. chacoense*, we have determined SGA content and composition in various tissues during a developmental time course. This wild potato species produces leptines, SGAs that are highly toxic to the Colorado potato beetle *Leptinotarsa decimlineata*, and has SGA levels that are at least 2-fold higher than the maximum target levels defined by breeders. The possibility that these two traits can be reconciled through introgression of *S. chacoense* with cultivated potato has driven considerable interest in this species. The plant also produces the two major SGAs associated with cultivated potato, a-chaconine and a-solanine, and additionally dehydrocommersonine. Our results indicate a structural diversity of SGAs in *S. chacoense* that is generated by combinations of three distinct glycosides and the formation of three major aglycones, leading to the accumulation of seven distinct SGA structures occurring in a tissue-specific manner.

THE SUBUNIT COMPOSITION OF HINOKIRESINOL SYNTHASE CONTROLS ENANTIOMERIC COMPOSITION OF HINOKIRESINOL FORMATION

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Norlignans are a class of phenylpropanoids with diphenylpentane carbon skeletons (C6-C5-C6) that are found mainly in conifers and monocotyledons. A norlignan, hinokiresinol, has an *E* or *Z* double bond in its molecule. Both hinokiresinols have antifungal activity and can be produced in response to stress, such as fungal infection and sapwood drying, suggesting that they are synthesized *in vivo* for plant protection.

Previously, our findings have indicated that (*Z*)-hinokiresinol was synthesized from a dimeric phenylpropanoid ester, 4-coumaryl 4-coumarate, by an enzyme preparation from elicitor-treated *A. officinalis* cells. In addition, (*E*)-hinokiresinol was formed from 4-coumaryl 4-coumarate by an enzyme prepared from *Cryptomeria japonica* (Japanese cedar) cells. Then, we purified (*Z*)-hinokiresonol synthase (HRS) from *A. officinalis*, and isolated cDNAs encoding the enzyme. The enzyme was found to be composed of two subunits (HRSα and HRSβ). When each recombinant subunit was incubated individually with 4-coumaryl 4-coumarate, only (*E*)-hinokiresonol was formed. On the other hand, incubating the same subatrate with a mixture of the two subunits gave rise to (*Z*)-hinokiresinol that is the geometric isomer accumulated in *A. officinalis*. Thus, the subunit composition can control the geometric isomerism of the product, which is quite interesting from organic chemical aspects.

Interestingly, in addition to the *cis-trans* regulation, the enantiomeric composition of hinokiresinol was found to be controled by the subunit composition of HRS. Thus, (*Z*)-hinokiresinol formed by the mixture of HRSα and HRSβ was optically pure (+)-enantiomer. It is noteworthy that the naturally occurring (*Z*)-hinokiresinol in *A. officinalis* is also optically pure (+)-enantiomer. In contrast, (*E*)-hinokiresinol formed following incubation with HRSα or HRSβ was not optically pure [(-) > (+), 20.6 or 9.0% enantiomer excess, respectively]. Therefore, it was demonstrated that the subunit composition of HRS controls not only geometric selectivity but also enantiomeric selectivity in hinokiresinol formation.

HPLC/MS PROFILING OF ANTHOCYANIN AND CAROTENES OF SWEET POTATO GENOTYPES WITH VARIABLE FLESH COLORS

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Sweet potato is one of top nutritional crops worldwide. A large number of varieties have been created for value-improved products, e.g. yellow and purple storage roots with high antioxidative effects. In this presentation, we report an application of HPLC/MS analysis for evaluation of anthocyanins and carotenoids of 20 sweet potato clones (varieties) with variable flesh colors including white, yellow, orange, and purple. Spectrophotometeric analysis at 530 nm showed dramatic variation of total anthocyanin levels from 0.43 mg/g dw to 1.36 mg/g dw among 8 purple fleshed genotypes. The genotype pur04-123 produces the highest level of total anthocyanins (1.36 mg/g dw). Yellow-purple flesh pur04-051, yellow flesh genotypes, and white flesh genotype L258, Won-MI, and Suwon 122 produce relative low levels of anthocyanins. Every purple sweet potato has a unique HPLC chromatography profile. More than 18 anthocyanins and 4 carotenoids were identified via LC-ESI-MS analysis. In addition, new anthocyanins identified from this research will be discussed. The method developed here for analysis of anthocyanins and carotenoids can be

used not only for nutritional evaluation of fresh sweet potato roots but also as an important tool for selection of sweet potato varieties.

FORMATION OF ALLYL/PROPENYL PHENOLS IN LIQUID MEDIA: SUBSTRATE VERSATILITY OF MONOLIGNOL ACYLTRANSFERASES AND ALLYL/PROPENYL PHENOL SYNTHASES

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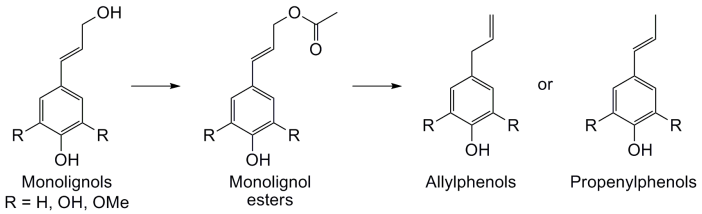
Monomeric allyl/propenyl phenols are important phenylpropanoid constituents of many plant aromas and flavours, e.g. in spices and herbs, and some find traditional medicinal uses as well, for example the anesthetic/antimicrobial eugenol from clove oil. Such compounds are thought to play a part within the defensive arsenal of plants, although in some cases they also serve as floral pollinator attractants. We are now pursuing opportunities for the massive production of these compounds for their potential use as petrochemical substitutes; i.e. allyl/propenyl phenols are typically liquids of high combustion energies, and are chemically related to styrenes, forming high-molecular weight polymers under appropriate reaction conditions.

As a first step towards the metabolic engineering of these biosynthetic pathways into selected plant species, we have demonstrated the capacity of genetically modified *E. coli* liquid cultures to produce allyl/propenyl phenols from the corresponding monolignols in acceptable yields. As part of this effort, we have isolated and characterized monolignol acyltransferases and allyl/propenylphenol synthases from *Larrea tridentata* (creosote bush) and *Piper regnellii* (pariparoba).

Here, we present our results regarding the bacterial production of allyl/propenyl phenols, and discuss the optimization of this bacterial system as a stepping stone towards engineering this pathway into tobacco and, ultimately, poplar. All the enzymes we have examined here display high substrate versatility, being able to efficiently process all the monolignols and monolignol acetates tested *in vitro*. Additionally, we will discuss the phylogenetic relationships between these proteins and other phenylpropanoid reductases.



Supported by NSF (MCB 0417291) and USDA (68-3A75-7-612).

THE SENSITIVITY OF HMBC SPECTRA USED FOR NATURAL PRODUCT STRUCTURE DETERMINATION BY NMR CAN DEPEND DRAMATICALLY ON PARAMETER CHOICES

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Two dimensional HMBC NMR spectra are of critical importance for determining structures of unknown natural products because they allow one to tie together molecular fragments into complete skeletal structures. However HMBC spectra are also the least sensitive of the set of 2D spectra used for structure determination. We demonstrate that, by appropriate choice of acquisition and processing parameters, one can get close to a ten-fold sensitivity increase for these spectra compared to results using parameters from a widely used text which suggests parameters for a wide range of NMR experiments.

POSTER PRESENTATIONS

POSTER 1

BIOASSAY-DIRECTED ISOLATION OF HYPOTENSIVE ALKALOID FROM *HOLARRHENA PUBESCENS*

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Holarrhena pubescens belongs to the family Apocynacea, commonly known as "kurchi" is highly reputed in traditional medicine as a remedy for amoebic dysentery and other intestinal ailment. Bioassay-directed fractionation [1] of the ethanolic extract of *Holarrhena pubescens* resulted in the isolation of steroidal alkaloids i.e. Holamide and Pubscinine.
Holamide showed a three proton doublet at 1.45 (J=6.56 Hz) and two AB doubles at 3.17 and 3.00 each for on proton (J=12.06 Hz) in the 1H NMR spectrum suggested that it belongs to conanine series of alkaloid (A class of compound with the steroid nucleus and a five members heterocyclic ring with nitrogen). In contrast Pubscinine showed one methyl at 1.28 while the doublet is missing a three proton singlet was observed at 2.28 due to a vinylic methyl indicated a double bond in the 18,20 – epimino ring of the conanine series of alkaloids.

In anaesthetized rats, the Holamide and Pubscinine caused a fall in blood pressure in a dose-dependent manner. Pretreatment of animals Atropine completely abolished the hypotensive response of Acetycholine; whereas hypotensive effect of Holamide and Pubscinine were not modified by Atropine [1]. Similarly Acetylcholine produced contractile effect in guinea-pig ileum, which was antagonized by atropine, however both (Holamide and Pubscinine) failed to produced any stimulant response on guinea-pig ileum. These data indicate that the steroidal alkaloids i.e. Holamide and Pubscinine from *Holarrhena pubescens* mediated hypotensive response through a mechanism different to that of Acetycholine.

1. Aftab, K., Shaheen, F., Mohammad, F. V., Noorwala, M., Ahmed, V. U. Phyto-pharmacology of saponins from *Symphytum officinale*, Adv Exp Med Biol 1996; 404: 429-442.

POSTER 2

A COMPARATIVE STUDY OF THE *IN VITRO* CYTOPROTECTIVE ACTIVITIES OF CANADIAN AND SIBERIAN POPULATIONS OF *RHODIOLA ROSEA*

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Rhodiola rosea L. (Arctic root, roseroot or golden root) is a widely known medicinal plant used in the circumpolar regions of Eurasia and recently discovered in the Nunavik region of Quebec, Canada. This shrub has been used traditionally to stimulate the nervous system, decrease depression, and enhance longevity, work performance and resistance to various emotional, physical and mental stressors. The phytochemical and pharmacological properties of the Canadian populations have yet to be studied, particularly with respect to their neuroprotective activities. Here, we describe a preliminary comparative study of Canadian (Nunavik) and Siberian (Eurasian) populations of *R. rosea* with respect to their cytoprotective activity against neurodegenerative endoplasmic reticulum (ER) stress pathways, which are prevalent in the cellular pathology of many diseases, including diabetes and Alzheimer and Parkinson's disease. Using rat adrenal pheochromocytoma cells (PC12-AC) as the cell model of interest and challenging them with two known ER stressors, thapsigargin and the calcium ionophore A23187, we show that crude ethanolic extracts of the two different populations contain specific cytoprotective activity, and act via different mechanisms to improve cell survival compared to controls. The subsequent identification and purification of active compounds using bioassay-guided isolation techniques will enable identification of potential plant-derived ER stress inhibitors for *in vivo* testing in animal models of human disease.

POSTER 3

ARTEMISININ BIOSYNTHESIS PROFILES IN *ARTEMISIA ANNUA* GROWING IN GROWTH CHAMBER

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Malaria is one of the top three devastating diseases in the world causing the loss of people's life. The parasite responsible for the vast majority of fatal malaria infections is *Plasmodium falciparum*, which can kill patients in only a few hours. The first natural product used to treat malaria was quinine. However, *Plasmodium falciparum* has developed resistance against this first antimalarial drug and also, more recently, to other related antimalarial drugs (chloroquine, mefloquine, primaquine, etc.). *Artemisia annua L.* (Qing hao) is an indigenous medicinal plant from China. In 1972 from the leaves of *A. annua*, a group of Chinese chemists isolated the natural product artemisinin, which is a novel sesquiterpene lactone highly active against *P. falciparum*. The content of artemisinin in *A. annua* is very low, in the range of 0.01-0.8% of dry weight of leaves dependent upon the growth regions. Certain progresses have been made in understanding the biosynthesis of artemisinin by these researchers; however, commercial production needs numerous additional efforts due to low yield. The main barrier to increasing the production of artemisinin is due to the unknown mechanism of the biosynthesis in plants. In order to overcome the barrier, we have optimized a growth condition to investigate the biosynthesis of artemisinin. In this study, we show a growth condition to effectively reduce the days of growth required for flower development. This growth condition allows the medicinal plant to be grown in a growth chamber like an *Arabidopsis thaliana* plant. The inflorescence development begins from the 15th node of most of plants when they are approximately 60-day's old. Seeds are mature for collection when the plants are nearly 80-day's old. HPLC-MS analysis was used to comparatively profile artemisinin and its precursors. The content of artemisinin from the 1st leaf to 17th leaf is gradually increased from a trace level to nearly 2 % in dry weight. The levels of artemisinin in the inflorescences developed from the 15th, 16th, and 17th nodes are nearly 2%. These preliminary data show that the biosynthesis of artemisinin is strongly plant development-dependent. In addition, from plants grown in growth chambers, we have already established a self-pollinated population for studying the biosynthesis of artemisinin and metabolic engineering of the antimalarial drug. The biosynthesis of artemisinin in the plants will be discussed in our presentation. This project is funded by North Carolina Biotechnology Center.

POSTER 4

ANALYSIS OF POTATO (*Solanum tuberosum*) CYP98A GENES

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The process of suberization has important implications in the wound-healing process in plants. Its formation has been shown to inhibit pathogen invasion and to prevent water loss. Suberin is a complex biopolymer comprised of a poly(aliphatic) domain (SPAD) similar to that of cutin and a poly(phenolic) domain (SPPD) similar to that of lignin. Formation of the SPAD and SPPD must be coordinately regulated by plants and must be deposited in a specific manner. Due to the similarities of the SPPD to lignin, it is important to identify a specific enzymatic step or reaction within general phenylpropanoid metabolism that acts exclusively within suberization in order to study the regulation of the suberization process. The conversion of *p*-coumaroyltyramine to caffeoyltyramine appears to be specific to suberization and could fulfill the role of a marker for wound-inducible suberization in potato. This unique step is catalyzed by *p*-coumaroyltyramine 3-hydroxylase (CYP98A), a cytochrome P450 that uses NADPH-reductase and oxygen to aid in the 3-hydroxylation of substrates involved in phenylpropanoid metabolism (e.g., *p*-coumaroyltyramine, *p*-coumaroylshikimate, *p*-coumaroylquinate, and 4-coumaroyl-4'-hydroxyphenyllactic acid). The goal of this research is to identify putative potato CYP98A genes, clone, sequence them and functionally characterize them. To date, five potential CYP98A genes have been identified in potato based on *in silico* analysis from an EST database. Gene specific primers have been designed, and tissue expression and suberin induction explored.

POSTER 5

PHYTOCHEMICAL SCREENING AND IMMUNOADJUVANT ACTIVITY OF *CALLIANDRA HAEMATOCEPHALA* EXTRACTS

Antony de Paula Barbosa, Bernadete Pereira da Silva, and José Paz Parente

Laboratory of Medicinal Plant Chemistry, Natural Products Research Nucleus, Health Sciences Centre, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Calliandra haematocephala (Leguminosae) is a native species found in Tropical America. This plant is widespread and cultivated with ornamental purposes in gardens and parks. Studies on the constituents of other species of this genus have been conducted in an attempt to isolate substances with immunomodulatory properties. However, a survey of the literature showed that no phytochemical investigations have been carried out on the constituents of this native species. In this work, we report the phytochemical screening and

the immunoadjuvant activity of the butanolic extract from the aerial parts of this plant. The phytochemical screening showed the presence of tannins, flavonoids and saponins in the butanolic extract. The immunoadjuvant property was evaluated against ovalbumin antigen, since the delayed type hypersensitivity reaction was measured as an *in vivo* assay of cellular immune response. Mice immunized with ovalbumin conjugated with extracts showed remarkable responses greater than those when the antigen was combined with commercial adjuvants. This response developed rapidly after immunization and persisted at lower levels for at least three days. The results obtained suggest the relevant adjuvant potential of the butanolic extract from *Calliandra haematocephala* in comparison with the commercial extract of *Quillaja saponaria*, a commonly used adjuvant for experimental vaccine formulations.

POSTER 6

NONDESTRUCTIVE ANALYSIS OF PHYTOCHEMICAL COMPONENTS BY NEAR INFARED (NIR) SPECTROSCOPY: MEASUREMENT OF ROSMERINIC ACID IN *PRUNELLA VULGARIS*

Mark A. Berhow, Brent Tisserat, Sandra Duval, Mark P. Widrlechner, and Candice Gardner
USDA, ARS, NCAUR
USDA, ARS, Plant Introduction Research Unit, Ames, IA

NIR spectroscopy has developed into a rapid nondestructive method to analyze in a single event an increasingly complex number of general and specific components in solid and liquid samples, including dissolved solids, acids, density, pH, microbial contamination, and percent oil, carbohydrate, protein, and moisture, as well as to determine concentrations of specific chemical components. The advent of rapid computer programs that utilize complex mathematical calculations, including Fourier transformation, has allowed for the computation of relationships between variation of multiple parameters and corresponding NIR spectra. In addition, the long pathlengths in NIR spectroscopy allow for sampling through glass and plastic, making NIR an even more efficient measuring process. When coordinated with proven chemical and physical analytical methods, the results of these standard chemical analyses can be translated into reliable NIR spectrometric calibrations. We have developed methods to measure the levels of the phenolic phytochemical rosmarinic acid, in dried leaf powders of Mentha and Prunella. The poster will discuss the extension of this methodology to rapidly measure a variety of phytochemical components in plant tissues and seeds. The long-term goal of this research is to provide rapid, large-scale analytical assessment of sample composition and nondestructively assess seed-compositional components for selection in breeding programs.

POSTER 7

GERMPLASM ENHANCEMENT FOR BLACK POD DISEASE RESISTANCE IN CACAO (*Theobroma cacao* L.)

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Black pod disease is caused by the fungus-like organism *Phytophthora*. It is the most widespread disease of *Theobroma cacao.* Of the seven known species of *Phytophthora* on cacao (*P. palmivora, P. capsici, P. citrophthora, P. megakarya, P. megasperma, P. nicotianae, P. arecae*) *P. palmivora* is the most predominant, being found almost everywhere cacao is planted. Losses from this disease have been estimated to be around 30% of average annual production. However, susceptible varieties and favorable environmental conditions can lead to losses as high as 90%. Chemical control of this disease is expensive, increasingly less effective and has significant environmental impact. Thus, developing plants with inherent resistance will affect progress in all the aforementioned areas. The Cocoa Research Unit, Trinidad and Tobago, has been part of a germplasm enhancement program initiated in 1998. The primary goal has been the accumulation of resistance genes to this disease in several diverse genetic groups derived from material housed at the International Cocoa Genebank, Trinidad. The program comprised two cycles involving evaluation of disease resistance in offspring derived from controlled crosses using a leaf disc method. Cycle 1 of the laboratory evaluations showed that 63.7% (of 3,486 seedlings) were moderate to highly resistant. This represented a considerable improvement from the parental genotypes. Field evaluations of the most promising types for black pod and witches' broom resistance, early flowering, vigor and pod index are currently in progress. Cycle 2 laboratory evaluations (involving crosses made from promising types of the first cycle) have just been completed. An increase in the number of resistant and moderately resistant types is expected. The most promising types in this cycle will be selected for field establishment and assessment.

POSTER 8

PUTATIVE SECONDARY PRODUCT GLUCOSYLTRANSFERASE EXPRESSION DURING *CITRUS PARADISI* GROWTH AND DEVELOPMENT

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Flavonoids are one of the most the abundant classes of secondary metabolites found in all plants. Several different kinds of flavonoid glucosyltransferases (GT) exist in the tissues of grapefruit *(Citrus paradisi)* making it a model plant for studying their structure and function. Efforts to understand how flavonoid GTs influence secondary metabolism and to study secondary product GT structure and function has led to the isolation of several putative secondary product GT clones (PGTs). This research was designed to test the hypothesis that PGT 2-8 are expressed constitutively during grapefruit seedling growth

and development. Alternatively, one or more could be expressed in a tissue-specific manner or developmentally regulated. We determined mRNA expression patterns of PGT 2-8 during the grapefruit seedling growth and development by quantifying mRNA expression levels in the roots, stems, leaves, and flowers using semi-quantitative RT-PCR and ImageJ was used to quantify intensity of bands standardized to 18s rRNA expression as a control. Six growth stages were defined. Findings show that there were variable degrees of PGT expression between the different tissues and stages of development. For example, a flavonol-specific 3-O-GT was expressed in nearly all tissues but with significantly higher levels in leaf tissue. PGT8, a clone with 98% homology to a limonoid GT, was not detectable in vegetative tissues or flowers, but was expressed in some fruit tissues. Additional results are presented. Therefore, results were more consistent with the alternative hypothesis that putative secondary product GT expression is tissue specific/and or developmentally regulated.

POSTER 9

BIOASSAY-DIRECTED ISOLATION OF HYPOTENSIVE ALKALOID FROM *HOLARRHENA PUBESCENS*

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POSTER 10

IS SABP2 A RECEPTOR FOR ACIBENZOLAR-S-METHYL (ASM): A CHEMICAL INDUCER OF SYSTEMIC ACQUIRED RESISTANCE (SAR) IN PLANTS?

Diwaker Tripathi [1], Yu-Li Jiang [2], Dhirendra Kumar [1]

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Plants defend themselves by developing an enhanced resistance to a broad spectrum of pathogens in the area of primary infection as well as in the distal, uninoculated parts. This induced state of resistance is called Systemic Acquired Resistance (SAR). Salicylic acid (SA), a plant hormone, is essential for the full development of SAR. Treatment of plants with SA makes them resistant to pathogens. SA-binding protein 2 (SABP2) is a methyl salicylate esterase that catalyzes the conversion of lipid mobile methyl salicylate (MeSA), synthesized in plants resisting pathogens, into SA. Plants which do not express SABP2 are unable to convert MeSA into SA and fail to develop an effective SAR response. Various functional analogs of SA have been developed and used to activate plants own natural defenses against microbial pathogens. ASM (Acibenzolar-S-Methyl) is one such functional analog which induces SAR in various families of monocot and dicot plants. ASM is commercially available by the name of BION and Actigard.

How ASM functions in plants is mostly unclear? We hypothesize that ASM activity depends on its conversion into an acid form, a reaction catalyzed by SABP2. We performed HPLC studies to show that SABP2 catalyzes the conversion of ASM ester into its acid form. To test our hypotheses in biological system, we are using transgenic tobacco (*Nicotiana tabacum* cv Xanthi nc) SABP2-silenced plants in which SABP2 gene expression is silenced by RNA interference and control plants, containing empty silenced vector. Using this system, we will determine if SABP2 catalyzed conversion of ASM ester into acid form is also required for (1) SAR induction and (2) expression of SAR genes. Results showing the action mechanism of ASM will be presented.

POSTER 11

CAROTENOGENESIS OF ORANGE COLOR IN CAPSICUM ANNUUM FRUIT

Ivette Guzman, Paul Bosland and Mary O'Connell

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Pepper, *Capsicum* spp., is a worldwide crop valued for heat, nutrition, and rich pigment content. Carotenoids, the largest group of plant pigments, function as antioxidants and as vitamin A precursors. The most abundant carotenoids in ripe peppers are β-carotene, capsanthin, and capsorubin. The genetic and biochemical basis for the orange color in peppers has not been fully explained. Carotenoid profiles and gene studies are lacking. A novel UPLC method was developed to efficiently separate 6 carotenoid standards with unique elution times. We have determined the carotenoid chemical profile present in 13 orange pepper varieties using UPLC; in some cases the orange appearance of the fruit was due to the accumulation of β -carotene, while in other cases, no β -carotene was detected, rather red and yellow carotenoids were the basis for the color. Four carotenoid biosynthetic genes, *Psy*, *Lcyb*, *Ca1*, and *Ccs* were cloned and sequenced from these cultivars. This data allowed us to test the hypothesis that different alleles for specific carotenoid biosynthetic enzymes are associated with specific carotenoid profiles in orange peppers. The results indicated polymorphic variation among the orange peppers analyzed. Supported in part by NM AES and NIH R25-GM61222.

POSTER 12

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF SUBERIN-ASSOCIATED OMEGA-HYDROXYLASES IN POTATO

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Suberin is a complex plant biopolymer that functions as a barrier against water loss and pathogen attack. Suberin biosynthesis involves the complex regulation of two metabolic pathways, lipid and phenolic metabolism, to form a macromolecule with two domains (poly(aliphatic) and poly(phenolic)) covalently linked together. In *Solanum tuberosum* L. (potato), 55% of the suberin poly(aliphatic) monomers are derived from the omega-hydroxylation of fatty acids, indicating that the omega-hydroxylase enzyme has a critical role in suberin formation. Omega-hydroxylases catalyze the hydroxylation of the terminal methyl group of fatty acids, and belong exclusively to the large protein family of cytochrome P450 enzymes. Thus far, two plant subfamilies have been identified as omega-hydroxylases, CYP86A and CYP94A. Members from each of these subfamilies have been shown to participate in cutin biosynthesis, but their role in suberin biosynthesis has not been investigated. In potato, a model system for studying suberin, three candidate omega-hydroxylase sequences have been identified through database searching. This study will

focus on the functional characterization of omega-hydroxylase sequences expressed in suberizing tissue, to determine if they are active in suberin biosynthesis. Functional characterization of the potato omega-hydroxylases will include testing for substrate specificity, enzyme kinetics and determining the molecular weight. Immunocytochemical localization of the potato omega-hydroxylases will be the first evidence for the location of suberin assembly, indicating whether the fatty acids are modified prior to- or post-incorporation into the macromolecule. Finally, exploration of the promoters of these omega-hydroxylases will lead to further understanding of the regulation involved in suberin biosynthesis. Understanding the process of suberin biosynthesis involves the characterization of a key reaction, omega-hydroxylation, which will lead to an increase in our basic understanding of the cooperative regulation of multiple metabolic pathways.

POSTER 13

GREENHOUSE STUDIES OF THE ALLELOPATHIC PROPERTIES OF SORGHUM (*SORGHUM BICOLOR*)

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Sorghum (*Sorghum bicolor* L. Moench) is an allelopathic species that represses weed growth through the exudation of the phytotoxic benzoquinone, sorgoleone. It has previously been shown that small-seeded weeds are most affected by sorgoleone, and redroot pigweed (*Amaranthus retroplexus* L.) has shown particular sensitivity to sorghum in previous studies. Two plant growth studies have been carried out to assess the impact of sorghum allelochemicals on neighboring plant growth. A target-neighbor design was used in which differing densities of pigweed were planted around a single sorghum plant. Pigweed was planted at densities of 0, 3, 6, 9, 12 plants per pot and the shoot dry mass was collected after 25 days. The expected result of resource competition in such a study would be that pigweed dry mass would decrease proportionately as the number of plants per pot increased. However, given the presence of phytotoxins produced by sorghum, deviations in this expected pattern may occur. Allelopathic effects are density-dependent, with greater effects observed at lower plant densities and reduced when plants share the available pool of toxins with one another. Thus allelopathic inhibition will be greatest at low pigweed densities, and resource competition most intense at high pigweed densities. This could then lead to maximum size of the pigweed neighbors occurring at an intermediate density. A second study was carried out to determine the potential impact of sorghum on the competitive outcome of two neighboring species which differ in their sensitivity to sorghum phytotoxins. In contrast to redroot pigweed, velvetleaf (*Abutilon theophrasti* Medic.) is less sensitive to the allelopathic effects of sorghum. In this study, four sorghum plants were established around a central zone containing either four pigweed plants, four velvetleaf plants, or two of each type. In half of the pots, the roots of the velvetleaf and pigweed target plants were kept separated from sorghum by planting

into Styrofoam cups buried in the pots, preventing root-root interaction between sorghum and the weed species. Given the greater sensitivity of redroot pigweed to sorghum, it is expected that the competitive balance between velvetleaf and pigweed will be shifted when the plants are grown in contact with sorghum. This is being assessed by measurement of shoot dry mass.

POSTER 14

UNRAVELING THE BIOSYNTHESIS OF VOLATILES IN PLANT DEFENSE: A MULTI-CATALYTIC *ARABIDOPSIS* CYTP450 ENZYME IS RESPONSIBLE FOR THE CONVERSION OF GERANYLLINALOOL TO THE INSECT-INDUCED HOMOTERPENE TMTT

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Volatile secondary metabolites such as homoterpenes released from vegetative plant tissues have significant functions in plant defense by directly warding-off herbivorous insects or attracting natural enemies of herbivores. This intriguing self-defense system has drawn increasing attention to the metabolic engineering of volatiles as a tool in developing alternative pest controls. The C_{16}-homoterpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) is emitted from aerial parts of many plants including crops such as maize, lima bean, tomato and alfalfa as well as the model plant *Arabidopsis thaliana.* TMTT emission is induced upon insect and mite attack and functions in attracting parasites or predators of these pests. Besides insect feeding, emission of TMTT can also be induced by fungal elicitors and bacterial pathogen infection.

We are interested in identifying the key enzymatic steps in the TMTT metabolic pathway. Here we demonstrate for the first time that CYP82G1 (At3g25180), a multi-catalytic CytP450 enzyme of the *Arabidopsis* CYP82 family, is responsible for the single-step conversion of the C_{20}-precursor geranyllinalool to TMTT. We describe that expression of the *CYP82G1* gene is coordinated with that of geranyllinalool synthase in *Arabidopsis* leaves upon treatment with the fungal elicitor alamethicin, particularly in a COI-1 dependent manner. TMTT emission is absent in *CYP82G1* loss-of-function mutants under elicitor-induced conditions but can be restored by complementation with the *CYP82G1* gene expressed under control of the constitutive CaMV35S promoter. Notably, *in vitro* enzyme assays with recombinant CYP82G1 and *in vivo* substrate-feeding assays in yeast cells over-expressing *CYP82G1* reveal a broader substrate specificity of the enzyme by converting not only geranyllinalool but also its C_{15}-analog nerolidol to the respective C_{11}-homoterpene DMNT. Histochemical *CYP82G1* promoter-GUS assays confirm inducible expression of this

gene in leaves upon insect feeding and show that constitutive expression is limited to stems and inflorescences.

POSTER 15

SEED COAT PHENOLICS AND THE DEVELOPING SILIQUE TRANSCRIPTOME OF YELLOW-SEEDED AND BROWN-SEEDED *BRASSICA CARINATA*

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Little is known about the molecular basis of the Brassica carinata dominant yellow seed, which is unique among the myriad of recessive yellow seed traits of other Brassica species. In order to address this deficiency, seed coats from near-isogenic B. carinata lines with brown seed or yellow seed were examined for more detailed differences in their phenolic compositions than previously determined. The structure of nine compounds, including four flavonoids, three lignans and two phenylpropanoids was elucidated on the basis of extensive 1D- and 2D-NMR spectroscopy, as well as ESI-MS-MS. The yellow seed coats accumulated flavonoids, phenylpropanoids and lignans, but soluble and insoluble proanthocyanidins were strongly reduced, while brown seed coats accumulated large amounts of both types of proanthocyanidins and phenylpropanoids and lignans equivalent to the amounts in the yellow seed, but lacked flavonoids. Both lines accumulated a mixed component containing quercetin and lignan derivatives and larger-sized unidentified material as their major HPLC-UV peak, but the peak was 0.4-fold lower in the yellow seed coats. DNA microarray analysis using a Brassica napus 15, 000 expressed sequence tag (EST) array indicated 296 developing silique genes which were differentially expressed more than 2-fold, including proteins involved in defense, metabolism, transcription, transportation, signal transduction, cytochrome P450, as well as proteins with unknown functions. Five genes (F3'H, FLS, F3H, FOMT and DFR) involved in the flavonoid pathway were differentially expressed, but genes in the phenylpropanoids pathway were not changed significantly. Regulatory factors and genes with unknown function were represented among genes with changed expression 2.4-fold more in developing yellow-seeded siliques than in brown-seeded siliques.

KEYWORDS: flavonoids, lignans, phenylpropanoids, B. carinata, microarray.

POSTER 16

CLONING AND EXPRESSION OF A PUTATIVE SECONDARY PRODUCT GLUCOSYLTRANSFERASE CLONE, PGT10, FROM *CITRUS PARADISI* USING *E. COLI*

EXPRESSION AND *AGROBACTERIUM*-MEDIATED TRANSIENT EXPRESSION IN *NICOTIANA TOBACUM*

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Flavonoids are a class of plant secondary metabolites that fulfill many functions including UV protection, producing pigmentation, having antipathogenic properties and acting as feeding deterrents. Glucosylation of flavonoids help flavonoids to perform their important roles *in planta* and these reactions are catalyzed by glucosyltransferases (GTs). The McIntosh lab research focus is on flavonoid metabolism and structure/function of secondary product GTs. *Citrus paradisi* (grapefruit) is used as the model plant system since it is very active in flavonoid metabolism and possesses a variety of secondary product GTs. This research is designed to test the hypothesis that PGT10 is a flavonoid glucosyltransferase. PGT10 was cloned into pCD1 vector which contains thioredoxin and 6His tags as well as a thrombin proteolytic cleavage site to remove these tags after recombinant expression. It was heterologously expressed and purified from *E.coli* extracts by immobilized metal ion affinity chromatography; however the majority of the expressed protein was found in insoluble inclusion bodies. In another approach, we are using an Agrobacterium-mediated transient expression system in *Nicotiana tobacum* which will likely overcome solubility issues. PGT10 has been subcloned into binary vector pER8, transformed into *Agrobacterium tumefaciens* GV3101, and transfected into *Nicotiana tobacum* leaves. Expression of the transgene in tobacco leaves was induced by estradiol treatment and PGT10 protein production is being monitored by western blot. If increased soluble PGT10 expression levels are observed, we will perform enzyme assays and rigorous biochemical analyses of the protein. The plant expression system will be evaluated for applicability in examination of other grapefruit PGTs.

POSTER 17

VARIABILITY OF CYP3A4 INHIBITION BY GINSENG ROOTS AND COMMERCIAL PRODUCTS

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Ginseng has been used in Traditional Chinese Medicine (TCM) to treat a variety of ailments including fatigue, insomnia, impotence, irritability, and even illnesses such as diabetes.

There is growing concern for the potential of herb-drug interactions with the use of ginseng, especially when taken with medications such as blood thinners and antidepressants. Cytochrome P450 (CYP) is the primary family of enzymes involved in the biotransformation and metabolism of drugs. In particular, CYP3A4 is known to metabolize more than 50% of clinically-prescribed drugs. This study was performed to determine if methanolic extracts of ground *Panax quinquefolius* and *Panax ginseng* roots, and commercial products have the potential to cause adverse effects through interactions with drugs by CYP3A4 inhibition using a high throughput fluorescence-based plate reader assay. Two different response profiles were obtained in the dose response study. The tested commercial products (Ontario Ginseng from Community Choice, Korean Ginseng, Red Chinese Ginseng from Swiss Natural Sources) had a weak inhibitory profile with IC_{50} values greater than 200 µg/mL. The ground root samples of Ontario-grown *Panax quinquefolius*, however, demonstrated a high potential to competitively inhibit CYP3A4 with IC_{50} values ranging between 34 to 44 µg/mL. Studies are underway to correlate inhibition potential with ginsenoside content through HPLC-DAD analysis. In particular, all samples will be characterized for their Rg1, Re, Rb1, Rc, Rb2, Rd, and Rf content. Overall, the results suggest that some ginseng products from the *Panax* genus may alter normal drug metabolism and consequently result in potential herb-drug interactions. Further studies are warranted to determine if these effects are clinically significant.

POSTER 18

HETEROLOGOUS EXPRESSION AND ELUCIDATION OF BIOCHEMICAL FUNCTION OF PUTATIVE FLAVONOID GLUCOSYLTRANSFERASE CLONES FROM *CITRUS PARADISI*

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Flavonoids are a major group of plant secondary metabolic compounds. Flavonoids play a variety of roles for plants including protection against UV light, pigmentation and flavor which can be important for pollination and seed dispersal, sexual reproduction and pollen development, antimicrobial and antifungal properties, and cues for microbial symbiont colonization. Flavonoids are also known to have benefit to human health due to antioxidant and anticancer properties. Glucosylation is a prominent modification reaction in flavonoid biosynthesis and these reactions are catalyzed by glucosyltransferases (GTs). *Citrus paradisi* (grapefruit) is an excellent model system for studying flavonoid GTs as it is known to contain GTs capable of producing flavanone, flavone, and flavonol 7-O-glucosides, flavonol 3-O-glucosides, and chalcone glucosides. Current efforts are focused on cloning, expression and characterization of putative GTs from grapefruit. This research is designed to test the hypothesis that grapefruit PGT2 and PGT9 clones are flavonoid glucosyltransferases. Results from experiments designed to optimize heterologous expression, enrich soluble recombinant protein, and screen for GT activity using a suite of

substrates are presented. Results show that PGT2 protein exhibits a low level of activity toward quercetin and the reaction product is being identified. PGT2 protein had no activity toward any of the other substrates tested. Results of work on PGT9, a clone obtained through a bioinformatic approach using the harvEST database, will also be presented.

POSTER 19

ALKALOIDS AS FEEDING DETERRENTS FOR GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.): A NEUROPHYSIOLOGICAL ANALYSIS OF GUSTATORY NEURON SENSITIVITY

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Alkaloids are secondary nitrogen-containing plant metabolites that generally have pharmacological effects. More than 3000 alkaloids have been identified and are common in more than 4000 plant species. Gypsy moth larvae, *Lymantria dispar* (L.), are highly polyphagous and display a wide host preference, feeding on the foliage of a few hundred species of plants. These larvae are major pest defoliators in the United States and destroy millions of acres of trees annually. They possess gustatory sensory organs located on the maxillae, namely the medial and lateral galeal styloconic sensilla. These sensilla play an important role in host-plant selection through the detection of phytochemicals, such as alkaloids. The styloconic sensilla each house four taste receptor cells within them, including a sugar, salt, deterrent, and inositol cell. Using a single cell electrophysiological tip-recording method, our aim was to characterize the temporal firing patterns and sensitivities of the receptor cells housed within each sensillum when exposed to a panel of selected phytochemicals. Our results revealed that these receptor cells responded to alkaloids, including strychnine, atropine, aristolochic acid, nicotine, and caffeine; sugars and sugar alcohols, including sucrose, fructose, and inositol; and salt, including potassium chloride. In general, the deterrent cell exhibited a robust temporal firing pattern and exhibited varying sensitivity to alkaloid stimulation. Thus, this study offers insights into the role of phytochemicals, especially alkaloids, in the taste physiology of this larval insect. It also provides a gateway for the possible use of alkaloids as antifeedants when designing biocontrol agents.

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POSTER 20

SPATIAL DISTRIBUTION AND DIFFUSION OF ROOT-EXUDED THIOPHENES FROM MARIGOLD (*TAGETES SPP.*)

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Roots of marigold (*Tagetes erecta* and *Tagetes patula*) are known to release the highly phytotoxic thiophenes 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) and α-terthienyl (α-T) into surrounding soil. Recently, solid phase root zone extraction (SPRE) probes constructed by inserting stainless steel wire into polydimethylsiloxane (PDMS) tubing have been shown to recover nano- to microgram quantities of thiophenes from soil beneath growing marigolds, using a 24 hour sampling time. The objective of these experiments was to extend these new methods to map the spatial distribution of thiophenes in the root zone. Marigolds were grown in a 1-cm thick layer of 1:1 sand/growth medium mix maintained between two foil-lined glass plates which were then clamped together. After plants were well-established, a thin, PDMS membrane was press-applied to the root zone and left in place for 24 hours. Membranes were then cut into 2.5 x 2.5 cm segments and analyzed for thiophene content. Marigolds were also grown in large PVC pipes with access ports drilled for insertion of SPRE probes at eight different depths. Following establishment of the marigolds, pipes were sampled weekly for three weeks to monitor thiophene content. Results thus far show that the amounts of thiophenes detected are highly variable, and do not directly correlate to the amount of root mass in adjacent soil. Amounts of BBT recovered with PDMS are often but not always greater than amounts of α-T measured. Furthermore, analyses of marigold roots show variable concentrations of thiophenes from one root portion to another, although amounts of BBT found are in general much higher than α-T. In order to gain insight into the behavior of thiophenes in soil, diffusion of α-T through agar and sand is being studied using PDMS probes loaded with known amounts of α-T. Diffusion of α-T has been observed over distances up to 5 cm in Phytagel agar, and up to 2 cm in sand. Further studies are continuing to explore reasons for the heterogeneity of thiophene distributions beneath marigolds.

POSTER 21

THE UTILITY OF COUPLED-HSQC SPECTRA FOR DETERMINING THE IDENTITIES OF SUGAR UNITS IN PROTON NMR-SPECTRALLY CROWDED SAPONINS

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Saponins are glycosides of steroids, steroid alkaloids or triterpenes found in plants, especially in the plant skins where they form a waxy protective coating. The structures of

three complex saponins, blighosides A-C from the tree *Blighia sapida*, were elucidated by one- and two-dimensional NMR and the H-1 and C-13 NMR spectra of these saponins assigned using only 1D and 2D NMR techniques. Blighoside A (MW 1086) has a tetrasaccharide linked to a triterpene aglycone, hederagenin. The four sugars in order of attachment at C-3 of hederagenin are arabinose, rhamnose, glucose, and another arabinose. Blighoside B (MW 1070) has the same tetrasaccharide linked to a different triterpene aglycone, oleanolic acid, which differs from hederagenin in that its C-23 is a methyl group rather than CH2OH. Blighoside C (MW 1504) has a hexasaccharide linked to oleanolic acid. The six sugars are attached at C-3 of oleanolic acid in the following linear array: two xyloses, rhamnose, glucose, a second rhamnose, and another glucose. Additionally, the first glucose is acetylated at positions 3 and 6 and the terminal glucose at positions 4 and 6. High-resolution coupled-HSQC spectra were especially useful for determining the identities of the monosaccharides in the three saponins. HMBC experiments enabled the linkages to be established between both the individual sugar units and C-3 of the two triterpenes. In all cases, complimentary 3-bond connectivities were observed between the anomeric protons and carbinol carbons and the respective anomeric carbons and carbinol protons. Both the H-1 and C-13 chemical shifts occur in relatively narrow spectral ranges, and the proton NMR spectra of all three saponins exhibit severe signal overlap. The coupled-HSQC spectra were of inestimable value in situations where protons on adjacent carbons had nearly coincident chemical shifts and the resulting strong H–H coupling made the measurement of vicinal couplings essentially impossible. Coupled-HSQC spectra elegantly obviated this problem because vicinal proton couplings are now determined from 1H – 13 C –12C—1H units, and the large one-bond C-H coupling effectively results in weak vicinal H–H coupling.

POSTER 22

PUTATIVE SECONDARY PRODUCT GLUCOSYLTRANSFERASE EXPRESSION DURING *CITRUS PARADISI* GROWTH AND DEVELOPMENT

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Flavonoids are one of the most the abundant classes of secondary metabolites found in all plants. Several different kinds of flavonoid glucosyltransferases (GT) exist in the tissues of grapefruit *(Citrus paradisi)* making it a model plant for studying their structure and function. Efforts to understand how flavonoid GTs influence secondary metabolism and to study secondary product GT structure and function has led to the isolation of several putative secondary product GT clones (PGTs). This research was designed to test the hypothesis that PGT 2-8 are expressed constitutively during grapefruit seedling growth and development. Alternatively, one or more could be expressed in a tissue-specific manner or developmentally regulated. We determined mRNA expression patterns of PGT 2-8 during the grapefruit seedling growth and development by quantifying mRNA expression levels in the roots, stems, leaves, and flowers using semi-quantitative RT-PCR

and ImageJ was used to quantify intensity of bands standardized to 18s rRNA expression as a control. Six growth stages were defined. Findings show that there were variable degrees of PGT expression between the different tissues and stages of development. For example, a flavonol-specific 3-O-GT was expressed in nearly all tissues but with significantly higher levels in leaf tissue. PGT8, a clone with 98% homology to a limonoid GT, was not detectable in vegetative tissues or flowers, but was expressed in some fruit tissues. Additional results are presented. Therefore, results were more consistent with the alternative hypothesis that putative secondary product GT expression is tissue specific/and or developmentally regulated.

POSTER 23

GINSENOSIDE VARIATION WITHIN AND BETWEEN ONTARIO-GROWN NORTH AMERICAN GINSENG (*PANAX QUINQUEFOLIUS*) POPULATIONS

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North American ginseng (*Panax quinquefolius*) is an increasingly important crop in Ontario. Cultivated Ontario ginseng populations have been growing separately for several decades with each population potentially possessing unique characteristics and phytochemical properties. Ginsenoside content and variation were assessed within and between five Ontario ginseng farm populations using HPLC-DAD. Ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd were quantified in 20-25 roots per population. Ginsenoside profiles were consistent across populations (Rb1> Rd> Re> Rc> Rg1> Rb2), which is unique in comparison with several reports of *P. quinquefolius* grown in other areas where Rd is often present in the lowest quantity. Significant variation was shown within each population, varying by 5-12% (w/w). There was no significant difference in mean total ginsenoside content between populations though there was a difference in Rc content between populations. This intra-population variation suggests potential for the development of cultivars with unique phytochemical profiles.

POSTER 24

SUBERIN LAMELLAE BIOSYNTHESIS IN *IRIS GERMANICA*'S MULTILAYERED EXODERMIS.

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More than 90% of angiosperm species have roots with an exodermis. Of these species, approximately 18% develop a multilayered exodermis (MEX), including roots of *Iris*

germanica. All of *I. germanica*'s mature MEX walls contain suberin lamellae with a poly(aliphatic) domain (SPAD) that is important for regulating radial water and ion transport. In general, the SPAD has a known monomeric composition as revealed from analysis of fully mature suberin lamellae. However, its synthesis in a maturing exodermis had never been examined. Our objective was to analyze SPAD biosynthesis by detecting and quantifying suberin-specific compounds at particular stages of MEX maturation and under different growth conditions. Roots were grown in hydroponic culture where the tank was either completely filled or partially filled to create a 60 mm humid air gap between the base of the rhizome and solution surface. MEX maturation was restricted in submerged roots but accelerated in root areas exposed to the air gap. At specific stages of maturation, the exodermis was isolated manually from the central cortex and flash frozen. Unpolymerized fatty acids, alkanes, and primary alcohols (soluble fraction) were isolated from the exodermal tissue using exhaustive micro-soxhlet extraction with chloroform:methanol (2:1). The remaining polymerized suberin monomers (insoluble fraction), including 18:1 α,ω-dioic acids and 18:1 ω-hydroxy fatty acids, were isolated by transesterification using 2 M MeOH/HCl at 80°C for 2 h. The isolated monomers from both the soluble and insoluble fractions were TMS derivatized, and then quantified and identified by GC-MS. The data to be presented will detail soluble and insoluble monomer compositions and amounts as the MEX matures in submerged or air gap-exposed root areas. Data collection over time revealed patterns of carbon flux into monomers, and allowed detection of monomers as they were actively incorporated into the SPAD.

POSTER 25

THE ANTICATARACTOGENESIS PROPERTIES OF TRADITIONAL VIETNAMESE AND CREE MEDICINES BY ALDOSE REDUCTASE INHIBITION.

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This research examines the role of natural products from traditional medicines as potential candidates in preventing the development of cataracts in type 2 diabetes by inhibition of aldose reductase. The focus of the study is traditional Vietnamese Medicinal (TVM) and Cree First Nation medicines used in treating symptoms of Type II Diabetes (T2D). Extracts of each plant were prepared by extraction with 80% ethanol and tested in an aldose reductase preparation obtained from pig eyes by monitoring NADPH consumption spectrophotometrically. *Glycyrrhizae uralensis* and *Cuscuta chinensis*, in particular exhibited over 90% inhibition of NADPH consumption at a concentration of 25 mg/ml of plant extract. Further analysis of these two plants revealed a linear dose-dependent relationship. The IC_{50} was determined to be $9.5\pm5x10^{-5}$µg extracts/mg protein and $5.5\pm5x10^{-5}$µg of

extracts/mg of protein for *C. chinensis* and *G. uralensis* respectively. Based on the positive findings with TVM plants, the current study is continuing the investigation of the potential of Cree medicinal plants in preventing cataractogenesis as a result of type 2 diabetes. A new microtiter plate method has been developed to increase efficiency of output.

POSTER 26

COMPARATIVE PHYTOCHEMICAL PROFILING OF TRADITIONAL WATER EXTRACTS AND ETHANOL EXTRACTS FROM CREE OF EEYOU ISTCHEE ANTI-DIABETIC BOTANICAL THERAPIES

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Six boreal forest plant species used by the Cree of Eeyou Istchee in the treatment of diabetes were analyzed phytochemically. The phytochemical profiles of standard laboratory ethanol extracts are compared with water extracts prepared using traditional methods. HPLC-DAD-ELSD and HPLC-DAD-MS were used to identify and quantify standards and isolated compounds. The majority of plant species investigated exhibited very similar phytochemical profiles, with only minor differences occurring in strongly polar and non-polar extremities of the chromatograms. However, important phytochemical differences are observed in one species where substantial qualitative and quantitative discrepancies between both types of extract are reported.

POSTER 27

ANTI-DIABETIC EFFECT OF ELEPHANT-FOOT YAM (*AMORPHOPHALLUS PAEONIIFOLIOUS* (DENNST.) NICOLSON) IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Elephant foot yam (*Amorphophallus paeoniifolious* (Dennst.) Nicolson) is a tropical tuber crop used in many ayurvedic preparations and is recommended in case of piles, dysentery, asthma, swelling of lungs and as blood purifier. The present investigation aims to study the anti-diabetic effect of elephant-foot yam. Dried powder of tuber was subjected to sequential extraction by using different solvents viz., hexane, chloroform, ethyl acetate, acetone and methanol. Among these, acetone extract exhibited highest antioxidant capacity by DPPH (IC_{50} value 8μg/ml) / Superoxide radical scavenging activity (IC_{50} value 62μg/ml), total phenol (866 mg GAE/gm of extract) and flavonoid (586 mg GAE/gm of extract) content. The effect of feeding acetone extract at 0.1 and 0.25% level in diet was studied in streptozotocin induced diabetic rats. The study involved a comparison between starch fed diabetic (SFD), acetone extract at 0.1% fed diabetic ($CFD_{0.1}$), acetone extract at 0.25% fed diabetic ($CFD_{0.25}$) and aminoguanidine fed diabetic (AFD) groups. Fasting blood sugar of $CFD_{0.1}$ and $CFD_{0.25}$ groups showed 23% and 37% reduction, respectively, whereas, AFD group showed 45% reduction in comparison to SFD group. Glomerular filtration rate of experimental rats in $CFD_{0.1}$ and $CFD_{0.25}$ groups showed 28% and 41% reduction, respectively, whereas, AFD group showed 54% reduction compared to SFD group. Amelioration of intestinal maltase activities by 18% 26% and 48% was observed in $CFD_{0.1,}$ $CFD_{0.25}$ and AFD groups respectively when compared to SFD group. Intestinal sucrase activity was high in SFD group and was ameliorated in $CFD_{0.1}$, $CFD_{0.25}$ and AFD groups to about 28, 45 and 56% respectively. Similarly, intestinal lactase activity was high in SFD group and was ameliorated in $CFD_{0.1}$, $CFD_{0.25}$ and AFD groups to about 36, 52 and 64%, respectively. In contrast decrease in renal maltase, sucrase and lactase were observed in SFD group while ameliorated in $CFD_{0.1}$, $CFD_{0.25}$ and AFD groups. The reduction in glycemic conditions may be attributed to antioxidant-rich phenols particularly flavonoids present in acetone extract. The results clearly indicated that acetone extract of elephant foot yam is an effective anti-diabetic agent to streptozotocin induced diabetic rats.

POSTER 28

ACTIVITY BASED FRACTIONATION OF GRAPE SEED PROANTHOCYANIDINS PERTAINING TO THEIR PROTECTIVE EFFECTS AGAINST ALZHEIMER'S DISEASE

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Grape seed extract (GSE) is rich in flavonoids, particularly proanthocyanidins (PAs) which are oligomeric and polymeric flavan-3-ols containing subunits of catechin, epicatechin and epicatechin gallate linked by C4-C8 or C4-C6 interflavan bonds. PAs and their monomers are strong antioxidants and have been ascribed a number of potential activities beneficial to health, including protection against cancers, cardiovascular disease and Alzheimer's

disease. However, little is known about the chemical nature and the concentration of bioactive polyphenols to which the body is exposed after ingestion of GSE.

We are investigating the activity based fractionation of grape seed extract into its respective monomers, oligomers and polymers in relation to their protective effects against Alzheimer's disease. Several techniques ranging from fractionation using liquid-liquid extraction, chromatography on Sephadex LH-20 and Toyopearl resin, (Normal/Reverse phase) HPLC and preparative HPLC have been optimized to separate and purify monomeric, oligomeric and polymeric PAs from grape seed extract. Each purified fraction will be used for bioavailability studies and investigation of effects on cognitive function in transgenic mice, through collaboration with researchers at Mt Sinai Medical Center (New York) and Purdue University. The extraction efficiency, together with the separation efficiency towards the resolution of monomers, oligomers and polymers with various techniques will be further discussed.

POSTER 29

BIOCHEMICAL AND MOLECULAR ANALYSIS OF THE FORMATION OF THE STRESS-INDUCED VOLATILE C_{11}-HOMOTERPENE (*E*)-4,8-DIMETHYLNONA-1,3,7-TRIENE IN THE ARABIDOPSIS ROOT

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Plants emit a mixture of volatile compounds in response to insect attack. These volatiles defend plants against herbivores through direct repellent activities and also function as indirect defense compounds by attracting natural enemies of herbivores. We investigate the formation of the acyclic C_{11}-homoterpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) which is a primary constituent of insect-induced volatile blends with important indirect defense activity (Kappers et al., 2005). Interestingly, DMNT is also emitted from roots of *Arabidopsis thaliana* after treatment with the defense hormone jasmonic acid (JA) suggesting defense functions of DMNT in plant roots. Our goal is to elucidate key enzymatic steps in DMNT biosynthesis and use this knowledge to genetically engineer DMNT biosynthesis in developing alternative plant-pest controls. Previous analysis of other plant species has demonstrated that DMNT is derived from the C_{15}-alcohol (*E*)-nerolidol. However, the enzymatic steps involved in the conversion of (*E*)-nerolidol to DMNT are not understood. We are using a combined biochemical and functional genomics approach to elucidate the biosynthetic pathway leading to DMNT formation. Our studies suggest a participation of cytochrome P450 enzymes in nerolidol degradation. Currently, few candidate CytP450 genes have been selected for screening of homozygous gene-knockout lines based on the analysis of publically available microarray datasets. Furthermore, we are investigating the subcellular organization of root-specific DMNT formation. Treatment with inhibitors such as lovastatin and fosmidomycin of the early cytosolic and plastidial terpenoid biosynthetic pathways, respectively, showed that DMNT formation is highly reduced only after lovastatin treatment, suggesting a major contribution of the cytosolic mevalonate pathway to DMNT formation.

POSTER 30

BIOCHEMICAL AND MOLECULAR ANALYSIS OF THE FORMATION OF THE STRESS-INDUCED VOLATILE C_{11}-HOMOTERPENE (*E*)-4,8-DIMETHYLNONA-1,3,7-TRIENE IN THE ARABIDOPSIS ROOT

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POSTER 31

GENE-METABOLITE RELATIONSHIPS IN BLACK COHOSH (*ACTAEA RACEMOSA* L)

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Black cohosh (*Actaea racemosa* L.) is a Ranunculaceae species native to the eastern parts of North America. Preparations from the rhizome and roots of black cohosh have been used medicinally by Native Americans to treat a variety of illnesses, including rheumatism and gynecological complaints. Today black cohosh is used chiefly as dietary supplement and non-estrogenic alternative to hormone-replacement therapy to alleviate menopausal vasomotor symptoms. However, very little is known about the active principles and about their biosynthesis in black cohosh. Initial results are presented from a study aimed at identifying gene content and diversity in *A. racemosa* and in an effort to pinpoint natural product genes for production of black cohosh signature compounds (triterpene glycosides and phenolic acids) and serotonergic agents (serotonin derivatives). cDNA libraries were constructed from young leaves and from rhizomes and roots of greenhouse-grown black cohosh plants and sequenced to obtain a collection of expressed sequence tags (ESTs). BLASTX analysis of 1500 ESTs from young leaves identified a large number of general metabolic genes (*e.g.*, for RuBisCo, ribosomal proteins, translation elongation factor 1-α), several genes encoding 2,3 oxidosqualene cyclase (cycloartenol synthase) an entry-point enzyme in triterpene (TT) biosynthesis, and BAHD-type acetyltransferases in phenylpropanoid (PP) biosynthesis. Among 400 rhizome/root ESTs, several gave matches to additional PP pathway enzymes (*e.g.*, hydroxycinnamoyl transferase). A black cohosh gene with similarity to genes for tryptophan decarboxylase—a key plant enzyme for the biosynthesis of serotonin—was cloned by degenerate PCR, enabling more detailed studies by *in vitro* protein expression of the encoded enzyme along with enzymes encoded by the candidate genes in TT and PP biosynthesis identified in the EST screens.

POSTER 32

ANTIOXIDANT ACTIVITY OF THE FRUIT PULP OF *ADANSONIA DIGITATA* (BAOBAB TREE)

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Baobab, *Adansonia digitata* L. (Malvaceae), is an unusually shaped tree native to Africa. In recent years, there has been increasing interest in developing baobab as a nutrient-rich botanical dietary supplement in the United States. The fruit or fruit pulp, the major parts of boabab used, contain various chemical components including amino acids, carbohydrates, flavonoids and other phenols, inorganic substances, lipids, organic acids, and vitamins.

From a bioactivity point of view, there are several reports on the effects of crude extracts and partially purified fractions of *A. digitata*, such as those having antioxidant, antibacterial, antifungal, anti-inflammatory, anti-sickling, and hepatitis C virus protease inhibition activities. The antioxidant activity has been tested by ABTS, DPPH, FRAP, photochemiluminescence, and trolox-based procedures, using various plant parts of baobab. As a result, undefined polyphenol and water-soluble flavonoid glycoside constituents have been implicated with antioxidant activity, but individual antioxidants have not been reported to date from *A. digitata.*

For the purpose of investigating the antioxidant components of baobab, our group has extracted the fruit pulp of baobab initially with methanol, and partitioned this dried extract with a series of solvents (hexane, chloroform, ethyl acetate, butanol and water) of increasing polarity. The antioxidant activities of these extracts were determined using a hydroxyl-radical scavenging activity test method, with 2′,7′-dichlorofluorescein as a probe. The ethyl acetate-soluble partition showed the most potent activity (ED_{50} 0.2 µg/ml) and this fraction was further fractionated by silica gel chromatography. From fraction BBE3 (ED_{50} 0.2 µg/mL) derived from this extract, several compounds have been isolated, and tested for antioxidant activity. Preliminary results of this investigation will be reported.

POSTER 33

INHIBTION OF GABA-TRANSAMINASE BY PIPERAMIDES: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR)

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The objective of this study was to investigate the structure-activity relationship of 25 natural piperamides and their synthetic analogs. An *in vitro* bioassay measuring GABA-transaminase activity in rat brain tissue was used. The piperamides were grouped based on the presence or absence of 3,4-methylenedioxyphenyl (MDP) and methoxy functional groups. QSAR models were generated using regression of log (1/Activity) as the dependent variable and either log partition coefficient (log P) or molar refractivity (MR) as the independent variable. Piperine, antiepilepsirine, and peepuloidin were most active with fifty percent inhibition (IC_{50}) values of 81 µM, 59 µM, and 79 µM, respectively. Structure-activity relationship analysis showed a significant positive correlation between the GABA-T inhibitory activity and the number of methoxy ($-OCH_3$) groups on the piperamides ($p < 0.001$; $r^2 = 0.404$). The best fit model was a quadratic function defined by the equations:

$$\text{Log } (1/\text{Activity}) = a\text{Log } P + b\text{Log } P^2 + c \quad (1)$$

$$\text{Log } (1/\text{Activity}) = aMR + bMR^2 + c \quad (2)$$

To our knowledge, this is the first QSAR report of piperamides and their effects on the GABAergic system, both of which could lead to interesting pharmaceutical leads.

POSTER 34

IS SABP2 A RECEPTOR FOR ACIBENZOLAR-S-METHYL (ASM): A CHEMICAL INDUCER OF SYSTEMIC ACQUIRED RESISTANCE (SAR) IN PLANTS?

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Plants defend themselves by developing an enhanced resistance to a broad spectrum of pathogens in the area of primary infection as well as in the distal, uninoculated parts. This induced state of resistance is called Systemic Acquired Resistance (SAR). Salicylic acid (SA), a plant hormone, is essential for the full development of SAR. Treatment of plants with SA makes them resistant to pathogens. SA-binding protein 2 (SABP2) is a methyl salicylate esterase that catalyzes the conversion of lipid mobile methyl salicylate (MeSA), synthesized in plants resisting pathogens, into SA. Plants which do not express SABP2 are unable to convert MeSA into SA and fail to develop an effective SAR response. Various functional analogs of SA have been developed and used to activate plants own natural defenses against microbial pathogens. ASM (Acibenzolar-S-Methyl) is one such functional analog which induces SAR in various families of monocot and dicot plants. ASM is commercially available by the name of BION and Actigard.

How ASM functions in plants is mostly unclear? We hypothesize that ASM activity depends on its conversion into an acid form, a reaction catalyzed by SABP2. We performed HPLC studies to show that SABP2 catalyzes the conversion of ASM ester into its acid form. To test our hypotheses in biological system, we are using transgenic tobacco (*Nicotiana tabacum* cv Xanthi nc) SABP2-silenced plants in which SABP2 gene expression is silenced by RNA interference and control plants, containing empty silenced vector. Using this system, we will determine if SABP2 catalyzed conversion of ASM ester into acid form is also required for (1) SAR induction and (2) expression of SAR genes. Results showing the action mechanism of ASM will be presented.

POSTER 35

EFFECTS OF AUXINS ON GENE EXPRESSION INVOVLED IN THE BIOSYNTHESIS OF ANTHOCYANINS IN PAP1-TRANSGENIC CALLUS

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PAP1 is a positive regulator of the biosynthetic pathway of anthocyanins. We have established red and white callus cultures of the PAP1-transgene. Our previous work reported that by varying the concentrations of 2, 4-D and NAA in the medium, the levels and profiles of anthocyanins in calli are affected. In this study, we report the effects of IAA, NAA, and 2, 4-D on gene expression controlling the biosynthesis of anthocyanin in PAP1-transgenic red calli. Transgenic white callus and wild-type callus were used as controls. Five concentrations of 0uM, 0.1uM, 1.0uM, 5.0uM, and 20.0uM for the tree auxins were

compared. Preliminary data showed significant different impacts of these concentrations on gene expression, biomass, and anthocyanin production. These data provide insight into the effects of auxins on the PAP1 transcription factor in the metabolic pathway. In this presentation, we will discuss the mechanism(s) of the effects of auxins on gene expression and the biosynthesis of anthocyanins. This research is funded by USDA-NRI (proposal number 2006-1334).

POSTER 36

ANALAYIS OF BIOACTIVE POLYPHENOLICS IN MUSCADINE GRAPES GROWING IN NORTH CAROLINA

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Muscadine grapes (*Vitis rotundifolia*) are a native wine grape species to the southeastern United States. Muscadine grapes are main resources for wine production in North Carolina. Muscadine grapes are approximately 1 to 1.5 inches in diameter, have thick skin, can produce up to five seeds per berry, and are known for their unique flavor and aroma. Muscadine grapes produce many potent antioxidative compounds (mostly found in seeds and skin) including: vitamins; phenolic acids; carotenoids; and flavonoids. Identified active compounds include anthocyanins, resveratrol, quercetin, gallic acid, ellagic acids etc. dependent upon cultivars. In this study, we interested in the production and structures of active polyphenolics e.g. condensed tannins and hydrolyzable tannins in two cultivars, Carlos and Nesbitt. Samples were collected from different developmental stages of flowers and berries. We are using LC-MS analysis to characterize the profiles of bioactive polyphenolics. In addition, we compare profiles of polyphenolics of the two cultivars with two *Vitis vinifera* cultivars, Cabernet Franc and Chardonnay. The data generated from this study will form a platform for genetic breeding or metabolic engineering to increase levels of bioactive polyphenolics, e.g. resveratrol and oligomeric condensed tannins, which can improve the quality of muscadine grape and wine products in North Carolina. This research is funded by North Carolina Wine and Grape Council..

POSTER 37

DEVELOPMENT-DEPENDENT FORMATION AND METABOLISM OF ANTHOCYANINS IN *ACER PALMATUM* SPECIES

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Anthocyanins are one of the richest pigments in plant kingdom. Their biological functions can be summarized as follows: antioxidative activity against oxidation-induced damage by

different environmental factors; attractive effects on pollinators, pathogen resistance etc. Over the past many years, numerous efforts have been made to determine the biosynthetic pathway of anthocyanins and also to identify several regulatory proteins mainly in flowers and fruits of model plants and crop plants. However, many questions concerning the metabolism of anthocyanins in foliage of flora remains unsolved. One example is "How candoes a developmental process impact on accumulation patterns of anthocyanins". In this study, we choose several cultivars from one of the most popular ornamental plants *Acer palmatum* to understand the mechanism of developmental changes of pigmentation in leaf. We propose that the metabolism of anthocyanins play an essential role in such changes. We used an integrated approach of phytochemistry, and metabolic profiling to determine the biosynthesis and metabolism of anthocyanins and their impacts on foliage color. Anthocyanins were extracted from different developmental stages of leaves for 6 *Acer palmatum* cultivars, which have obviously different accumulation patterns of anthocyanins in spring. Spectrometric analysis showed three major development-dependent trends of anthocyanin levels in leaves growing in spring: 1) gradual decrease; 2) constitutive accumulation; 3) lacking anthocyanins. HPLC-MS analysis showed that the alteration of anthocyanin levels resulted from either qualitative or quantitative change or from both changes, while qualitative changes probably stem from structure changes or modification or degradation. Proanthocyanidin analysis has beencarried out as well to determine their relationship with both anthocyanin production and foliar coloration. We have found that even for green leaves with no/trace amount of detectable anthocyanins, the biosynthetic pathway of anthocyanidin/proanthocyanidin is still activated. And our results indicate that metabolic channeling directing the anthocyanin pathway to the proanthocyanidin biosynthesis plays a very important role in pigmentation pattern change along developmental processes. (This project is supported by Polk County, North Carolina.)

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POSTER 38

PREDICTABLE TRANSITIVE RNA INTERFERENCE INDUCED BY MRNA HAIRPINS IN *C. ELEGANS*

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The specific silencing of genes through RNA interference (RNAi) by double-stranded RNA is now being studied not only as a means to understand cellular processes, but also as a means of treating patients in the pharmaceutical industry and controlling pests in the agricultural sector. While the protein machinery necessary to cause this phenomenon has been well characterized, it is not always possible to predict the silencing of one specific gene. At times, when a target gene is silenced, it can lead to the silencing of other RNAs. We are trying to understand this process, termed transitive RNAi, and trying to determine if there are scientific rules governing it. Using a computer algorithm we developed, we have been able to create maps of gene networks in which such interactions might occur due to sequence homology. One of these networks is now being examined in the nematode

Caenorhabditis elegans. Two plasmid constructs have created. The first targets four genes within the network for silencing through a common hairpin sequence found in each of the genes. The second construct targets just one these genes through sequence homology in an area outside of the hairpin. The two constructs, as well as a control construct, have been introduced into bacteria, induced to express the dsRNA and fed to the *C. elgans* to induce RNAi. Nematodes fed the first construct are phenotypically very similar to nematodes fed the second construct. Both exhibit impaired movement as compared *C. elegans* fed the control construct or no construct. This suggests that the pattern of silenced genes in similar between the two groups and that the silencing of the one gene with the second construct might have led to the silencing of the other genes in manner that we could then begin to predict. We are currently examining RNA levels for all of the genes with the *C.elegans* network to determine if there is a discernible pattern with rules that we can determine.

POSTER 39

ON THE ORIGIN OF METABOLITE MODULARITY AND ARRAYS IN PLANT SPECIALIZED METABOLISM

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Many plants, such as ginger, turmeric, sweet basil and tomato and related species, produce arrays of related secondary or specialized metabolites. Sweet basil produces a variety of methylated flavones, tomato and related Solanum species produce a multitude of acyl sugars. Turmeric produces dozens of diarylheptanoids related to the medicinally active curcumin. Ginger produces not only a large array of diarylheptanoids, but biogenetically related gingerols and other related compounds as well. All of these species also produce diverse collections of terpenoids, as well as compounds from other metabolic pathways. How these large arrays of compounds are produced and how their production is controlled remain largely unanswered questions, although recent progress is beginning to shed light on these questions. One feature of specialized metabolism that has emerged recently is that it appears to be organized at times and in certain species (if not all) in a modular fashion. That is, groups of metabolites that are biosynthetically linked can accumulate in a concerted manner, in biosynthetic modules. The existence of these modules suggests concerted control of production of such compounds. In the case of the species listed above, the large collection of related metabolites that accumulate do not all belong to the same module. Indeed, subgroups of compounds from these species accumulate in separate modules. This suggests that multiple enzymes must be involved in production of these compounds. Identification of such enzymes and characterization of their individual functions are important steps in understanding how such complex arrays of metabolites can evolve in specific plant lineages.

POSTER 40

PROMOTER ANALYSIS OF POTATO (*Solanum tuberosum*) FATTY ACID w-HYDROXYLASE (FA ω H1)

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Suberin is a plant biopolymer that provides a protective barrier between its underground organs and the external environment, preventing water and nutrient loss and providing protection against pathogen attack. Suberin is composed of two different polymeric domains; the poly-aliphatic domain (SPAD) and the poly-phenolic domain (SPPD). Suberization is induced by wounding, and requires the coordinate biosynthesis of new aliphatic and phenolic components. With respect to the aliphatics, newly synthesized fatty acids can undergo one of two main metabolic fates: (1) the elongation of stearic acid (18:0) to very long chain, saturated fatty acids (VLCFA – C20 to C28), with and without the further modification to *n*-alkanes and 1-alkanols and (2) the oxidation of oleic acid (18:1) to produce ω-hydroxy-fatty acids (18-OH-18:1) and α, ω -dioic acids. The conversion of oleic acid to an ω-hydroxy-fatty acid is a metabolic step unique to suberin (and cutin) formation, and therefore can be used as a marker to study SPAD formation in tissues that suberize. This reaction is catalyzed by members of two different cytochrome P450 families; CYP86A and CYP94A, and are referred to as fatty acid ω -hydroxylases (FA ω H). In *S. tuberosum* one member of the *CYP86A* family (FA ω H1) has been shown to have high levels of expression in suberizing root tissue. In the present study, we have sequenced a portion of the FAwH1 promoter region. Using *in silico* analyses, putative regulatory motifs have been identified in the FA ω H1 promoter that may control its expression.

POSTER 41

IDENTIFICATION AND CHARACTERIZATION OF AN ANTHOCYANIDIN/FLAVONOL 3-O-GLUCOSYLTRANFERASE GENE ISOLATED FROM THE SEED COAT OF BLACK SOYBEAN

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The seed coats of black soybean (*Glycine max* (L.) Merr.) are known to accumulate all anthocyanins required for the red (cyanidin 3-O-glucoside), blue (delphinidin 3-O-glucoside), purple (petunidin 3-O-glucoside), and orange (pelargonidin 3-O-glucoside) coloration of plant tissues[1]. Black soybean anthocyanins have recently demonstrated a range of medicinal activities such as anti-inflammatory[2], anti-obesity[3], anti-apoptotic[4] and anti-proliferative[2] activities, and to enhance immune response[5]. Almost all structural genes involved in anthocyanin biosynthesis in soybean have been characterized[6-12], but the gene required to catalyze the final step, namely anthocyanidin 3-O-glucosyltransferase, remained to be identified. In this study we have isolated an anthocyanidin/flavonol 3-O-glucosyltransferase from the soybean seed coat. Complementation of anthocyanin biosynthesis in an *Arabidopsis thaliana* mutant was used to verify the *in vivo* function of the soybean gene. The recombinant enzyme used only anthocyanidins and flavonols as substrates *in vitro*. Cyanidin, but not kaempferol, inhibited recombinant enzyme activity at levels above 12 μM. This property may function to limit cytoplasmic anthocyanin levels or flux into the anthocyanin pathway *in vivo*. UDP-galactose could also be used as a sugar donor at low velocity relative to UDP-glucose. Thus, high levels of glucosylated and low levels of galactosylated anthocyanins in the seed coat of black soybean may be attributed to the activities of a single enzyme.

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