PSNA News

Phytochemical Society of North America
Sociedad Fitoquímica de América del Norte
Société Phytochimique de L'Amerique du Nord

Volume 46, Number 1 July 2007

From the President Norman G. Lewis

The hour is rapidly approaching: The next PSNA annual meeting will be held in the Donald Danforth Plant Science Center from July 21-25, 2007. I am delighted—and hope that everyone is—with the quite fantastic range of scientific topics to be covered. These of course reflect the remarkable scientific diversity of the PSNA membership, and the field of phytochemistry in its many forms and in its allied fields. The program for this year's annual meeting, the short biographies of the invited speakers, and the abstracts follow the newsletter below. The progress made in restructuring some of the PSNA's activities is also described. See you in St. Louis!

2007 PSNA Annual Meeting Venue: The Donald Danforth Plant Science Center (DDPSC)

We are particularly grateful to Roger Beachy, President, DDPSC, for agreeing to have their wonderful Center serve as the venue for this year's meeting. Conference delegates will have the added treat of being shuttled from the hotel to the DDPSC for the various technical sessions—for many, this will remind you of your early childhood days—as you will learn!

Donald Danforth Plant Science Center

The DDPSC was founded in 1998 as a non-profit research institute with a main focus being on improving the human condition through plant science. Since its opening in 2001, some of its important missions include basic and applied research, education and training, with goals of feeding the hungry, improving human health, preserving and renewing the environment, as well as helping address the area of renewable energy through biofuels. At the meeting, you will also learn more about how effective Dr. Beachy has been in putting into place a large and successful group of interdisciplinary researchers to help achieve these noble goals.

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WWW.PSNA-ONLINE.ORG

The Phytochemical Society of North America

The Phytochemical Society of North America (PSNA) is a nonprofit scientific organization whose membership is open to anyone with an interest in phytochemistry and the role of plant substances in related fields. Annual membership dues are U.S. $40 for regular members and $20 for student members. Annual meetings featuring symposium topics of current interest and contributed papers by conference participants are held throughout the United States, Canada, and Mexico. PSNA meetings provide participants with exposure to the cutting-edge research of prominent international scientists, but are still small enough to offer informality and intimacy that are conducive to the exchange of ideas. This newsletter is circulated to members to keep them informed of upcoming meetings and developments within the society, and to provide a forum for the exchange of information and ideas. If you would like additional information about the PSNA, or if you have material that you would like included in the newsletter, please contact the PSNA Secretary or visit our website at www.psna-online.org. Annual dues and changes of address should be sent to the PSNA Treasurer. Also check the PSNA website for regular updates.

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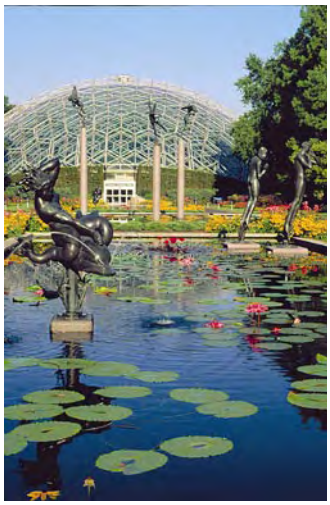
DDPSC Atrium

We are also particularly delighted that Dr. William H. Danforth, Chancellor Emeritus of Washington University and chairman of the Board of Directors of the Donald Danforth Plant Science Center, will give the Opening Remarks at the PSNA annual meeting. We look forward to learning more of his personal reflections and insights into the vision of the Danforth family, i.e. how this independent plant research institute came about and which is dedicated to the various compelling problems facing humanity. We look forward to knowing more about where plant science can—and will—help on such matters. Dr. Danforth will also touch upon yet another of his favorite topics, namely progress towards a new federal competitive funding entity for plant science that is based, to some extent, on the NIH model.

More information about DDPSC can be found on its website at http://www.danforthcenter.org/.

Missouri Botanical Garden, a National Treasure

We are also delighted to have the opportunity to visit the Missouri Botanical Garden, particularly on Henry Shaw's birthday. Originally from England, Henry Shaw (July 24, 1800 – August 25, 1889) became a highly successful St. Louis businessman—however, he is best remembered as the founder of the Missouri Botanical Garden and for his life-long interests in botany. Beginning first as his estate, the Garden was later opened to the general public in 1859.



Missouri Botanical Garden

Today, the Missouri Botanical Garden is now one of the truly great botanical gardens in the world. Led by its President, Peter H. Raven, it has become a focal point in helping improve our understanding of biodiversity and of the fragility of our diverse living systems and environments. We are particularly pleased that Dr. Peter Raven will bring us up-to-date with the challenges that we (humanity) face with the stewardship of biodiversity on this planet.

From a research perspective, the Missouri Botanical Garden is also a most important scientific resource. Many scientists, including myself, have frequently been helped enormously in gaining access to plant specimens worldwide. For example, we are indebted to the assistance by MBG personnel and their associates worldwide in legally securing hard-to-obtain plant specimens/plant germplasm resources, etc.

More information about the MBG can be found on its website at http://www.mobot.org/.

PSNA Annual Meeting Scientific Program

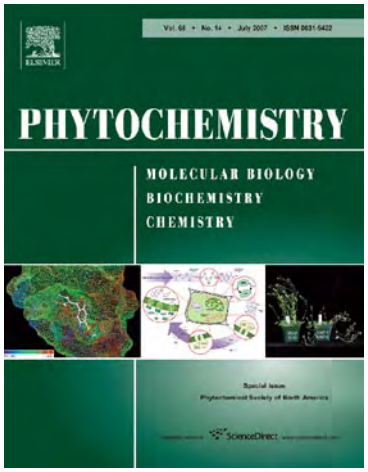
This year's scientific program organized by Daneel Ferreira, Toni Kutchan and myself, builds further upon your desire to maintain a Gordon Conference-like format for our meeting—and where the diverse areas of phytochemical research are represented. This year, the meeting will host scientists from many different countries: Austria, Brazil, Canada, Denmark, Germany, Italy, Japan, Korea, Mexico, Nigeria, Thailand and the United States. We are delighted to have such an international gathering, and encourage each of you to make new friends and acquaintances.

We also hope that you enjoy reading the biographies of our invited speakers and learning more about their varied scientific interests.

PSNA Awards Banquet

Building upon the history of the Society, this year's banquet will follow in the same vein as last year's. That is, the best student and postdoctoral papers will be selected and recipients announced at the banquet, together with the student travel awards. We will also be pleased to recognize our younger researchers through the Arthur C. Neish Young Investigator Awards. This year's banquet will additionally honor the PSNA Phytochemistry Pioneers through the Phytochemistry Pioneer Awards; this particular form of recognition was initiated in 2006.

Phytochemistry Special Issue: 2006 PSNA Conference



Following the 2006 PSNA Annual Meeting, we decided to experiment with alternate publication routes from that of the highly successful Recent Advances in Phytochemistry series. To that end, various researchers were invited for contributions to the first PSNA Special Issue of *Phytochemistry*. This Special Issue of *Phytochemistry* is now in print (Volume **68(**14); Pages 1821-2022), and includes the following articles:

Authors (Corresponding Au.)	**Manuscript Title**	**Pages**
David G. I. Kingston	The shape of things to come: Structural and synthetic studies of taxol and related compounds	1843-1853
J. E. Brandle, P. G. Telmer	Steviol glycoside biosynthesis	1854-1862
Patrick S. Covello, Keat T. Teoh, Devin R. Polichuk, Darwin W. Reed, Goska Nowak	Functional genomics and the biosynthesis of artemisinin	1863-1870
Lukasz Kutrzeba, Franck E. Dayan, J'Lynn Howell, Ju Feng, José-Luis Giner, Jordan K. Zjawiony	Biosynthesis of salvinorin a proceeds via the deoxyxylulose phosphate pathway	1871-1880
Sergio Martínez-Luis, Araceli Pérez-Vásquez, Rachel Mata	Natural products with calmodulin inhibitor properties	1881-1902
Sanja Roje	Vitamin B biosynthesis in plants	1903-1920
Mary Magnotta, Jun Murata, Jianxin Chen, Vincenzo De Luca	Expression of deacetylvindoline-4-*O*-acetyltransferase in *Catharanthus roseus* hairy roots	1921-1930
Michaël Jourdes, Claudia L. Cardenas, Dhrubojyoti D. Laskar, Syed G.A. Moinuddin, Laurence B. Davin, Norman G. Lewis	Plant cell walls are enfeebled when attempting to preserve native lignin configuration with poly-*p*-hydroxycinnamaldehydes: Evolutionary implications	1931-1955

Authors (Corresponding Au.)	Manuscript Title	Pages
Sung-Jin Kim, Kye-Won Kim, Man-Ho Cho, Vincent R. Franceschi, Laurence B. Davin, Norman G. Lewis	Expression of cinnamyl alcohol dehydrogenases and their putative homologues during *Arabidopsis thaliana* growth and development: Lessons for database annotations?	1956-1973
Steven G. Ralph, Sharon Jancsik, Jörge Bohlmann	Dirigent proteins in conifer defense II: Extended gene discovery, phylogeny, and constitutive and stress-induced gene expression in spruce (*Picea* spp.)	1974-1990
Fabricio Medina-Bolivar, Jose Condori, Agnes M Rimando, John Hubstenberger, Kristen Shelton, Sean F O'Keefe, Selester Bennett, Maureen C Dolan	Production and secretion of resveratrol in hairy root cultures of peanut	1991-2002
Franck E. Dayan, Stephen O. Duke, Audrey Sauldubois, Nidhi Singh, Christopher McCurdy, Charles Cantrell	*p*-Hydroxyphenylpyruvate dioxygenase is a herbicidal target site for β-triketones from *Leptospermum scoparium*	2003-2013
James D. McChesney, Sylesh K. Venkataraman, John T. Henri	Plant natural products: Back to the future or into extinction?	2014-2021
Gail Shadle, Fang Chen, M. S. Srinivasa Reddy, Lisa Jackson, Jin Nakashima, Richard A. Dixon	Down-regulation of hydroxycinnamoyl CoA: Shikimate hydroxycinnamoyl transferase in transgenic alfalfa affects lignification, development and forage quality	Vol. **68**(11): 1521-1529*

* Article published in earlier issue of *Phytochemistry*.

Advances in Plant Biochemistry and Molecular Biology (APBMB)

The first volume of a new series entitled Advances in Plant Biochemistry and Molecular Biology (APBMB) is now being published, also having Elsevier as its publisher. Volume 1 is entitled "Bioengineering and Molecular Biology of Plant Pathways", which now begins immediately following the completion of RAP series, and will be an annual offering. It is planned to be an ISI-tracked publication series, i.e. with total citations/impact factors coming online when it has run for a few years. Volume 1 contains 13 chapters, and was edited by Hans J. Bohnert, Henry T. Nguyen and Norman G. Lewis. The series will also build upon recommendations from a recent NSF Workshop entitled "Realizing the Vision: Leading Edge Technologies in Biological Systems."

Future Meetings

Year 2011 will mark the 50th anniversary of PSNA; at the business meeting this year, plans for the PSNA meetings 2008, 2009, 2010 and 2011 will be discussed. So please attend the business meeting for further information.

PSNA Membership

A substantial effort was put into place this—and last—year to consolidate and expand upon the current PSNA membership. I am pleased to inform you that the membership drive so far has built the numbers back up again to a healthy (and increasing) membership level. We have many more new members to recruit, however, and I ask each of you to continue to help get the word out.

Other Restructuring

The business meeting will also update the membership on the activities of the Committees now in place, including the Newsletter/website committee. The revised Constitution will also be presented.

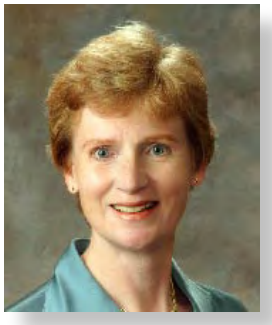
Invited Speakers

Ed Cahoon

Dr. Cahoon is an Associate Member at the Donald Danforth Plant Science Center in Saint Louis, Missouri USA. He has conducted research in the area of in seed oil metabolism for the past 17 years. Dr. Cahoon has also conducted research in soybean biotechnology, including soybean transformation, for the past eight years as a research scientist and principal investigator at DuPont Crop Genetics as well as a research molecular biologist with the United States Department of Agriculture-Agricultural Research Service. He is the author of over 40 research papers and reviews and an inventor on 16 issued U.S. Patents. Dr. Cahoon's research has blended aspects of biochemistry, molecular biology, and functional genomics to improve the fatty acid composition of oilseeds for food, feed, and industrial uses. His work has resulted in the identification of the biosynthetic pathways for a number of unusual fatty acids from non-agronomic plant species with potential commercial value. These pathways were subsequently transferred by Dr. Cahoon and colleagues to seeds of genetically enhanced crops, including soybean, to create oils with novel properties. More recently, Dr. Cahoon has expanded his research to the study of isoprenoids with emphasis on the tocotrienol form of vitamin E. He has applied this research toward the enhancement of the nutritional value and antioxidant content of oilseeds and toward the biofortification of cassava, as a member of the BioCassava Plus consortium.

Jon Clardy

Jon Clardy grew up in the Virginia suburbs of Washington, DC. He attended Yale University with the intention of majoring in anthropology and going to medical school but fell in love with organic chemistry. He received a Ph.D. from Harvard University in organic chemistry and began his independent career at Iowa State University. After eight years there, he moved to Cornell University where he was a member of the faculty for twenty-five years. He moved to Harvard Medical School in 2002 where he is a member of the Biological Chemistry and Molecular Pharmacology faculty, directs the Chemical Biology Graduate Program, and co-directs the Infectious Disease Initiative at the Broad Institute of Harvard and MIT. His research has always involved, in one way or another, the small biologically active molecules produced by nature, and his current research emphasizes ways to find new molecules using DNA-based approaches, chemical signaling and ecology in genetically accessible organisms, and infectious disease. He enjoys teaching – most recently a course for non-science major undergraduates in Harvard College entitled The Molecules of Life and in the introductory graduate courses offered by the Chemical Biology Program.

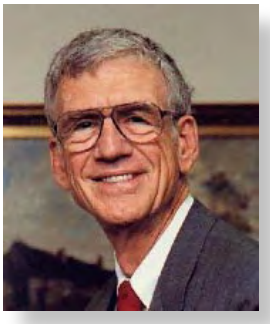


Katrina Cornish

Senior Vice President, Research & Development, Yulex Corporation

Dr. Katrina Cornish is the leading U.S. scientific expert and is internationally recognized as a principal authority on guayule, guayule latex production, and on natural rubber biosynthesis in general. Prior to joining Yulex in 2004, Dr. Cornish led the USDA's development of domestic natural rubber and rubber latex sources for >15 years. Dr. Cornish is the sole inventor of process and product patents to produce safe natural rubber latex and products from guayule. She began working closely with Yulex in 1997 when the company first licensed her discoveries from the USDA. She has over 110 publications and patents (not including abstracts), of which more than 85 are related to rubber biosynthesis and production. Amongst her numerous awards, her guayule latex development was recognized by the Connect 2005 Most Innovative New Product Award,

to Yulex for yulex™ latex in the Category Life Sciences – Medical Devices & Diagnostics. In 2004, Cornish received the Good Housekeeping Award for Women in Government in recognition of her inspired leadership and proving that good government can change our lives. In 2002, she was elected a Fellow of the American Association for the Advancement of Science and won a presidential award from the American Chemical Society. In 1998, she was honored with the USDA's Scientist of the Year Award for Outstanding Senior Research Scientist. In 1997, she was recognized by the Agricultural University of Antonio Narro, Mexico, for guayule research. Dr. Cornish received a first class honors degree in biological sciences and a Ph.D. in plant biology from the University of Birmingham, England.



William H. Danforth

Dr. William H. Danforth is currently Chancellor Emeritus of Washington University. He chairs the Coalition of Plant and Life Sciences and is chairman of the Board of Directors of the Donald Danforth Plant Science Center.

Born in St. Louis, Missouri, on April l0, l926, he received his B.A. from Princeton University and his M.D. from Harvard Medical School in l95l. After completing his internship in medicine at Barnes Hospital, St. Louis, Missouri, he served in the United States Navy from l952-54. He returned to St. Louis to continue medical training at Barnes Hospital and at St. Louis Children's Hospital.

Dr. Danforth joined the Washington University Medical School faculty in l957. In l967, he was appointed Professor (internal medicine), which is his present faculty rank at the University. From l965-7l, he served as Vice Chancellor for Medical Affairs and as President of the Washington University Medical Center. He became Washington University's thirteenth Chancellor July l, l97l and served until his retirement June 30, l995. He also served as Chairman of the Board of Trustees of Washington University from July l, l995 to June 30, 1999. Dr. Danforth is a member of the Institute of Medicine and served on the Council l977-79.

Dr. Danforth was appointed to chair the Research, Education and Economics Task Force for the USDA by Agriculture Secretary Ann M. Veneman in 2003. He is a Director on the Board of Trustees of the Danforth Foundation and is a Trustee of the American Youth Foundation. He co-chaired the Board of Directors of Barnes-Jewish Hospital and served on the Boards of Directors of Ralston Purina Company, BJC Health System and Energizer Holdings, Inc.

Birgit Draeger

Birgit Draeger is a professor at Martin Luther University Halle-Wittenberg. She studied pharmacy, food chemistry, and plant biology at the University of Muenster, Germany. Her PhD studies in plant biochemistry and plant biotechnology were completed under the supervision of Professor Wolfgang Barz, in 1986, with a thesis on phenylpropanoid metabolism of photoautotrophic and photomixotrophic cell cultures of *Chenopodium rubrum*. Thereafter, Birgit Draeger was appointed a postdoctoral research fellow at the Research Center for Cell and Tissue Culture of Kyoto University in Japan in the laboratory of Professor Yasuyuki Yamada and Professor Takashi Hashimoto, where she joined in on studies on tropane alkaloid biosynthesis. In 1995, she received her Habilitation and *venia legendi* for Pharmaceutical Biology from Muenster University. She was later appointed a full professor of Pharmaceutical Biology at Halle University in 1996. Her research areas are medicinal natural compounds, as well as biochemistry and molecular biology of plants. She currently teaches plant secondary product metabolism, plant genetics, and plant biotechnology. One of her research goals is directed toward obtaining an understanding of tropane alkaloid biosynthetic steps, the regulation of their biosynthesis, the evolution of tropane metabolism, and the roles that tropane alkaloids have *in planta.* Apart from tropanes, she also works on benzyltetrahydroisoquinoline alkaloids, on the triterpene betulinic acid, on lignans, and on plant-derived vitamins.

A. Douglas Kinghorn

Since May 2004, Prof. A. Douglas Kinghorn has been Jack L. Beal Professor and Chair in Natural Products Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University. Formerly, he was Professor of Pharmacognosy, Assistant Head of the Department of Medicinal Chemistry and Pharmacognosy and Associate Director of the Program for Collaborative Research in the Pharmaceutical Sciences at the University of Illinois at Chicago (UIC).

Dr. Kinghorn was educated in the United Kingdom, and received degrees from the Universities of Bradford [B. Pharm. (Special) in Pharmacy, 1969], Strathclyde (M.Sc. in Forensic Science, 1970), and London [Ph.D. in Pharmacognosy, 1975; D.Sc. (earned higher doctorate) in Pharmacy, 1990]. He received postdoctoral training at the University of Mississippi (1975-1976) and at the University of Illinois at Chicago (UIC; 1976-1977). At UIC, he was then appointed as Assistant Professor (1977-1981), Associate Professor with tenure (1981-1986), and Professor (1986-2004). Dr. Kinghorn is a Fellow of the Linnean Society of London (1985), the Royal Pharmaceutical Society of Great Britain (1991), the American Association of Pharmaceutical Scientists (AAPS) (1996), the American Association for the Advancement of Science (AAAS) (2006), and of The School of Pharmacy, University of London (2006). He was designated as the 1993 B. Kenneth West University Scholar (Senior University Scholar) by the University of Illinois Foundation and was awarded the 2002-2003 UIC Award for Excellence in Teaching.

Dr. Kinghorn's research has been supported by the U.S. National Institutes of Health (NIH) and by private industry. His research interests are on the isolation, characterization, and biological evaluation of natural products of higher plant origin, and he has worked on antimicrobials, cancer chemopreventive agents, cancer chemotherapeutic agents, and noncariogenic sweeteners. Since 1988, he has served as a frequent ad hoc NIH grant reviewer, and was a member of the NIH AIDS and Related Diseases D Study Section during the period 1993-1997. He is currently Chair of the Dietary Supplements – Botanicals Expert Committee (2005-2010) of the United States Pharmacopeia.

Dr. Kinghorn has served as President of both the American Society of Pharmacognosy (1990-1991) and the Society for Economic Botany (1991-1992). Currently, he is Editor-in-Chief of the *Journal of Natural Products* (1994-2008; co-published by the American Chemical Society and the American Society of Pharmacognosy) and serves on the Editorial or International Advisory Boards of about 15 other scientific journals. He has authored or co-authored about 400 research articles, book chapters, and reviews, and has edited or co-edited four books, and been assigned as co-inventor in nine patents. In 2001 he was named as a "Highly Cited Researcher" in Agricultural Sciences by the Institute of Scientific Information (ISI), Philadelphia, Pennsylvania. Dr. Kinghorn has been Major and/or Thesis Advisor to about 40 graduate students and has also directly supervised about 60 postdoctorals and visiting scholars.

Kent F. McCue

Dr. Kent F. McCue is a Research Geneticist in the Crop Improvement and Utilization Research Unit at the USDA Agricultural Research Service's Western Regional Research Center in Albany, CA. Dr. McCue received his B.A. in Biology from Harvard University and his Ph.D. in Plant Physiology from the University of California at Davis. He did post-doctoral research at the Michigan State University Plant Research Laboratory, the UC Berkeley/USDA Plant Gene Expression Center, and the USDA Western Regional Research Center. During his postdoctoral research, Dr. McCue utilized a variety of disciplines to understand and manipulate complex biochemical pathways and biochemical adaptations to environmental stress including light and fungal elicited phenylpropanoid protectants, salt and drought inducible betaine osmoprotectants, heavy metal inducible phytochelatin protectants, and environmental and developmental regulation of starch production. Dr. McCue is currently a Scientist on a project entitled "Improved Molecular Genetic Tools for Potato Improvement". His primary project is to control the accumulation of steroidal glycoalkaloids (SGAs) in potato tubers. SGAs are bitter, undesirable, compounds that accumulate in potatoes during storage, spouting or after injury. SGAs are a food safety issue when levels exceed specified levels and can hinder efforts to introduce desirable traits from genetically diverse wild potato relatives. The goal is to identify the genes that code for enzymes responsible for the synthesis of SGAs and use these genes as tools to suppress accumulation of SGAs. Dr. McCue has elucidated the function of three genes

encoding Steroidal Alkaloid Glycosyl Transferases (SGT) involved in the biosynthesis of SGAs. These genes are being used to manipulate accumulation and the relative abundance of the two major SGAs found in potatoes, solanine and chaconine. Other projects include the utilization of transgenes to improve resistance of potatoes to viral, bacterial and fungal pathogens and to address post-harvest disorders.

Debra Mohnen

Debra Mohnen is Professor in the Department of Biochemistry and Molecular Biology and at the Complex Carbohydrate Research Center (CCRC) of the University of Georgia (UGA). She is also an adjunct faculty member of the Department of Plant Biology, a member of the Plant Center at UGA, and Lead on Plant Cell Wall Biosynthesis Research in the Bioenergy Science Center. Dr. Mohnen received her Ph.D. degree in Plant Biology from the University of Illinois, Urbana, with research conducted at the Friedrich Miescher Institute in Basel, Switzerland. Her research, funded by NSF and USDA, centers on understanding the biosynthesis, function and structure of the family of plant cell wall polysaccharides known as pectins. More recently her research has also moved into studies on hemicelluloses and on walls as sources of lignocellulosic biomass for biofuel production. Following in depth biochemical studies of pectin synthesis, she identified the first gene expressing an enzymatically proven pectin biosynthetic glycosyltransferase, galacturonosyltransferase 1 (GAUT1) and the GAUT1-related gene family proposed to encode enzymes involved in pectin and xylan synthesis. Her current research is focused on elucidating the biochemical pathways and mechanisms of pectin and cell wall synthesis and using this information to decipher the biological functions of wall polysaccharides in plants and in human health. Dr. Mohnen has served on many scientific panels and currently serves as invited faculty sponsor for the UGA Association for Women in Science (AWIS). She has given numerous invited presentations at international meetings on plant cell wall structure, function and synthesis and is Chair-elect of the 2009 Plant Cell Walls Gordon Research Conference. As Co-PI on the NSF-funded *"*Plant Cell Wall Biosynthesis Research Network*"* she also established and runs "CarboSource Services", an NSF-funded service that provides rare substrates for plant wall polysaccharide synthesis to the research community. She is active in the American Chemical Society Cellulose and Renewable Materials Division where she served as past member-at-large and current Secretary-elect.

Birger Lindberg Møller

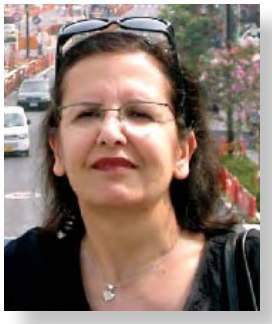
Birger Lindberg Møller (BLM) obtained his Ph.D. in plant biochemistry and organic chemistry in 1975 from the University of Copenhagen. The topic for his Ph.D. thesis was biosynthesis and degradation of lysine in plants. He spent the following three years as a Fulbright Fellow at Professor Eric Conn´s Laboratory, University of California, Davis working on the elucidation of the biosynthetic pathway for cyanogenic glucosides. In the period 1977-1984, BLM was employed as Senior Scientist and Niels Bohr Fellow at the Department of Physiology, Carlsberg Laboratory, with Professor Diter von Wettstein working with the light reactions of photosynthesis. His D.Sc. thesis in 1984 is based on this work. In 1984, he was appointed Research Professor at The Royal Veterinary & Agricultural University (now merged with the University of Copenhagen) and in 1989 Full Professor in plant biochemistry. In 1998, he was also appointed as Director of Center for Molecular Plant Physiology (PlaCe), the Danish "center of excellence" within plant science founded by the Danish National Research Foundation. A main research interest is biosynthesis, transport, storage and degradation of cyanogenic glucosides and elucidation of their role in the ability of plants to communicate with the environment and to defend themselves against attack from herbivores and pests and abiotic stress. The knowledge obtained is used to improve the nutritive value of important crop plants and to improve their disease resistance by classical plant breeding as well as by genetic engineering.

Professor Møller is a member of the Danish Board of Patent Appeals, a member of the Board of the Danish plant breeding company Pajbjerg, and a member of the Scientific Advisory Boards of the biotech companies Aresa and Evolva. He is also member of the Board of Trustees of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, of the Board of the Leibniz Institute, Halle, Germany and of the Advisory Board of Institut de Biologie Moléculaire des Plantes (IBMP), CNRS, Strasbourg, France. He has been elected

member of the Royal Danish Academy of Science and Letters and is a very active participant in the public debate in Denmark and Europe concerning the use of gene technology in food production. Professor Møller has published more than 220 scientific contributions to international journals, and throughout his career has developed a tight and mutually beneficial collaboration with major Danish and international industries with the purpose of getting his research accomplishments implemented in production of new improved products. He has been awarded several research prizes, including the Villum Kann Rasmussen Research Prize (500,000 US$) in 2007, the largest Danish research award.

Andrés Navarrete

Andrés Navarrete is professor in the Pharmacy Department, Chemistry School, of the Universidad Nacional Autónoma de México, México City, México. He has over twenty-five years´ experience in the research of pharmacology and chemistry of medicinal plants and has authored forty one original scientific articles on the identification of active principles of Mexican medicinal plants. These include description of the mechanisms of action and development of analytical methods for analysis of raw material and commercial herbal products. He has graduated three Ph.D., eight M. Sc. and forty B.Sc. students, and is a member of the Mexican United States Pharmacopoeia Committee of Experts- Natural Products. He contributed importantly to the edition of the Mexican United States Herbal Pharmacopoeia. Dr. Navarrete has been distinguished with several awards in Mexico, one of the most important of which is the Martín de la Cruz National Health Award in 2001. Dr. Navarrete has lectured in National Meetings in Mexico and taught numerous pharmacology courses in several Mexican Universities. He has also has organized several conferences on quality control of herbal medicines and pharmacology of medicinal plants.



M. Soledade C. Pedras

M. Soledade C. Pedras was born in Portugal where she obtained B.Sc. and Lic. degrees from the University of Porto. She came to Canada with a NATO fellowship to study Organic Chemistry in the Department of Chemistry, University of Alberta, from which she graduated with a Ph.D. degree in 1986. She then accepted a postdoctoral Canadian Government Laboratory Visiting Fellowship at the Plant Biotechnology Institute, National Research Council Laboratory, in Saskatoon where she stayed as a researcher until June 1994. She then accepted a faculty position in the Department of Chemistry at the University of Saskatchewan, where she is a full professor since July 1998. She is currently a Tier 1 Canada Research Chair in Bioorganic and Agricultural Chemistry.

Dr. Pedras sees chemistry as a tool to understand life processes at the molecular level. Combining this interest with a very strong liking of plants led her to work in the interactions of plants with their pathogens. Her studies involve the determination of the chemical mediators of the interaction between plants (crucifers) and pathogens, and application of this information to understand and predict the disease resistance of plants and control virulence of plant pathogens. The great chemical challenge of this work involves the discovery of metabolites with important roles in keeping plants healthier in their natural or cultivated environment and in designing and producing novel selective crop protection agents with minimal environmental impact. Her projects include biosynthetic pathways of phytoalexins, phytoanticipins and phytotoxins, isolation of detoxifying enzymes and design and synthesis of inhibitors of metabolic processes specific to fungi.

Peter H. Raven

Peter H. Raven is Director of the Missouri Botanical Garden and one of the world's leading botanists and advocates of conservation and biodiversity. In addition, he is past President and Chairman of the Board of the American Association for the Advancement of Science, the largest organization of professional scientists in the world. Dr. Raven is also Chairman of the National Geographic Society's Committee for Research and Exploration, and Chair of the Division of Earth and Life Studies of the National Research Council, which includes biology, chemistry, and geology.

For three decades, Dr. Raven has headed the Missouri Botanical Garden, an institution he nurtured to a world-class center for botanical research, education, and horticulture display. Under Dr. Raven's leadership, the Missouri Botanical Garden has become a leader in botanical research in Latin America, Africa, and Asia, with strong programs in North America as well. The Garden's education program in the St. Louis region reaches more than 100,000 students each year and provides professional development for teachers. The splendid horticultural displays attract more than 750,000 visitors to the Garden annually, including tourists to St. Louis from around the United States and the world. He is also the Engelmann Professor of Botany at Washington University in St. Louis.

Described by TIME magazine as a "Hero for the Planet," Dr. Raven champions research around the world to preserve endangered plants and is a leading advocate for conservation and a sustainable environment. In recognition of his work in science and conservation, Dr. Raven is the recipient of numerous other prizes and awards, including the prestigious International Prize for Biology from the government of Japan; Environmental Prize of the Institute de la Vie; Volvo Environment Prize; the Tyler Prize for Environmental Achievement, and the Sasakawa Environment Prize. He has held Guggenheim and John D. and Catherine T. MacArthur Foundation Fellowships.

He was a member of the President's Committee of Advisors on Science and Technology during the Clinton Administration. In 2001, he received from the President of the United States the National Medal of Science, the highest award for scientific accomplishment in this country. Dr. Raven served for 12 years as Home Secretary of the National Academy of Sciences, is a member of the academies of science in Argentina, Brazil, China, Denmark, India, Italy, Mexico, Russia, Sweden, the U.K. and several other countries and the Pontifical Academy of Sciences. He was first Chair of the U.S. Civilian Research and Development Foundation, a government-established organization that funds joint research with the independent countries of the former Soviet Union, and served as President of the XVI International Botanical Congress in St. Louis in 1999.

Dr. Raven is Co-editor of the Flora of China, a joint Chinese-American international project that is leading to a contemporary account on all the plants of China. He has written numerous books and publications, both popular and scientific, including Biology of Plants (co-authored with Ray Evert and Susan Eichhorn, Worth Publishers, Inc., New York), the internationally best-selling textbook in botany, now in its sixth edition, and Environment (Saunders College Publishing, Pennsylvania), a leading textbook on the environment.

Dr. Raven received his Ph.D. from the University of California, Los Angeles, in 1960 after completing his undergraduate work at the University of California, Berkeley. He has received honorary degrees from universities in this country and throughout the world. February 2003 (N)

Kazuki Saito

Professor Kazuki Saito was born in Nagano in 1954, Japan. He graduated from the Faculty of Pharmaceutical Sciences, the University of Tokyo, Japan in 1977 and then obtained his Ph.D. for bio-organic chemistry/biochemistry of pharmaceutical sciences from the University of Tokyo in 1982. After staying in Keio University in Japan and Ghent University in Belgium (Prof. Marc Van Montagu's laboratory), he began molecular biology and biotechnology of plant primary and secondary metabolism in Chiba University. After being appointed as a lecturer and an associate professor, he was promoted to Full Professor in 1995 at the Graduate School of Pharmaceutical Sciences, Chiba University, Japan, where he has remained until now. Since 2002, he has also been a Special Guest Researcher in Kazusa DNA Research Institute in Kisarazu. Since April, 2005, he was also appointed as a Group Director at RIKEN Plant Science Center in Yokohama to direct the Metabolomics Group and Metabolic Function Group. He has published more than 170 original papers and 70 invited reviews and book chapters. His research interests are metabolome-based functional genomics, biochemistry, molecular biology and biotechnology of primary and secondary metabolism in plants. In particular, he is engaged in the biosynthetic studies of sulfur compounds, flavonoids, quinolizidine and indole alkaloids He is also interested in music, movies, detective novels and Bon-Sai.

Fumihiko Sato

Professor, Department of Plant Gene and Totipotency, Graduate School of Biostudies, Kyoto University, Kyoto, Japan.

Born in 1953, Dr. Satoh obtained a bachelor's degree in 1975 and a Ph.D. in 1981 at Kyoto University under Prof. Yasuyuki Yamada. Employed first as an instructor at the Faculty of Agriculture, Kyoto University in 1979, then as an Associate Professor in 1995, he was promoted to Full Professor in 1997, before taking up his present position in 1999. Dr. Satoh also worked with Prof. Wolfgang Barz at Muenster University, Germany, as a Humboldt postdoctoral fellow from 1983 to 1984. Throughout his career, he became interested in "functional differentiation of plant cells" as a cell culture specialist and later as a molecular and cellular biologist. His interests range from photoautotrophic tobacco cells and transgenic plants, chloroplast development, gene expression in cultured cells, a chloroplast-nucleoid-DNA-binding protease (CND41), senescence, biogenesis of photosynthetic apparatus, especially the oxygen evolving complex including PsbP, and stress adaptation. Functional differentiation of secondary metabolism, especially isoquinoline alkaloid biosynthesis and its metabolic engineering, are other main research subjects of his laboratory. Using high-berberine producing *Coptis japonica* cells, which were established in 1980 by cellular selection, almost all biosynthetic genes in berberine biosynthesis from tyrosine have been isolated and characterized to dissect the regulation mechanism of cellular differentiation in plants. Endless research continues to understand the totipotency of plant cells. Dr. Satoh is an Editor for several journals including Plant Cell Report and Plant and Cell Physiology (2004-). He has received the Scientific Contribution Award from the Ichimura Foundation in 2003 for "Development of novel production systems for useful plant-derived alkaloids using isoquinoline alkaloid biosynthesis as a model".

David Shintani

David Shintani received his Bachelors of Science Degree in Genetics from U.C. Davis in 1985. Upon graduation, he worked as a research technician at Calgene in Davis, California. David then went on to receive his Ph.D. with John Ohlrogge in the Department of Botany and Plant Pathology at Michigan State University where he studied regulation of fatty acid biosynthesis in plants. As a postdoctoral fellow, he next worked with Dean DellaPenna to decipher the Vitamin E biosynthetic pathway. In 2000, he was hired in the Department of Biochemistry at the University of Nevada where his research foci are the areas of rubber and thiamin biosynthesis in plants.

Paul Talalay

Paul Talalay, M.D. is John Jacob Abel Distinguished Service Professor of Pharmacology and Molecular Sciences at Johns Hopkins University School of Medicine. He holds the S.B. degree in Biophysics from M.I.T. and the M.D. degree from Yale. Following surgical training at the Massachusetts General Hospital, he moved to the University of Chicago, rising to the academic ranks of Professor of Biochemistry, Professor of Medicine, and Professor in the Ben May Laboratory for Cancer Research. After serving for 12 years as Director of the Department of Pharmacology at Johns Hopkins Medical School, he relinquished this position to devote himself full time to research.

Dr. Talalay has devoted his career to cancer research. For the last 25 years, he has been involved in devising strategies for chemoprotection against the risk of cancer, a field in which he is recognized as a pioneer. His efforts have focused on achieving protection by raising the enzymes concerned with the detoxication of carcinogens. Analysis of the chemistry and the molecular biology of boosting enzymes of detoxication has led him and his colleagues to devise simple cell culture methods for detecting chemical and especially dietary phytochemicals that raise these enzymes. This work led to the isolation of sulforaphane as the most potent inducer of protective enzymes in broccoli. These findings led to the organization of the *Brassica Chemoprotection Laboratory at* Johns Hopkins. This unique laboratory is exclusively dedicated to identifying edible plants that are particularly rich in protective enzyme-inducer activity.

Dr. Talalay's honors, in addition to his appointment as a University Distinguished Service Professor, include: appointment to one of the first life-time Professorships of the American Cancer Society; Membership in the National Academy of Sciences, in the American Academy of Arts and Sciences, and in the American Philosophical Society. He was recently awarded the Linus Pauling Institute Prize for Health Research. He has published approximately two hundred and fifty papers in internationally respected scientific journals. He has received an honorary D.Sc. degree from Acadia University, and the M D.-Ph.D. Student Library at Johns Hopkins has been named in his honor.

Brenda S. J. Winkel

Brenda S.J. Winkel is a Professor of Biological Sciences at Virginia Tech, where she has been on the faculty since 1992. Dr. Winkel earned a B.S. in Chemistry and a M.S. in Biochemistry from Southern Illinois University and a Ph.D. in Genetics from the University of Georgia. She started working on flavonoid metabolism in Arabidopsis in 1989, when she joined the laboratory of Howard Goodman at Harvard Medical School and Massachusetts General Hospital as a postdoctoral fellow. The advent of yeast two-hybrid technology in the early 90's led her to begin exploring the organization of the flavonoid pathway as a "metabolon" of interacting enzymes. This has been the primary focus of her research for the past 15 years, which has applied a wide range of technologies in an effort to understand the intracellular organization and localization of flavonoid metabolism at the molecular level. The work has incorporated the use of tools such as electron and confocal microscopy, biophysical methods such as surface plasmon resonance and isothermal titration calorimetry, and structural biology, including the use of small angle neutron scattering and molecular modeling. Recent evidence for the nuclear localization of several of these enzymes is leading to a new model for flavonoid enzymes in plants that involves a "moonlighting" role in the nucleus, the current research focus in the lab. Dr. Winkel also has a long-standing collaboration with the Brewer laboratory in the Department of Chemistry to develop new multimetallic anti-cancer agents that bind to DNA. She also recently helped establish a Molecular Plant Sciences graduate program at Virginia Tech and is the current Director of an IGERT program entitled, "Exploring Interfaces in Graduate Education and Research," which is aimed at promoting interdisciplinary research across engineering and the sciences.

Zheng-Hua Ye

Zheng-Hua Ye received his B.S. degree in Biology from Fudan University, China (1983), his M.S. degree in Plant Physiology from the Institute of Botany, Chinese Academy of Sciences (1988), and his Ph.D. degree in Plant Biology from Washington University in St. Louis (1994). His graduate and postdoctoral work with Professor Joseph E. Varner ranged from expression analysis of cell wall structural proteins, molecular characterization of xylem differentiation and lignification to genetic study of fiber differentiation and vascular patterning. He joined the Department of Plant Biology, University of Georgia in 1996 as an Assistant Professor and he is currently an Associate Professor in Plant Biology. He has been using fibers as a model to dissect the molecular mechanisms underlying cell differentiation, cell elongation and secondary wall formation. Current work in his lab focuses on study of the biosynthetic pathway of xylan, the second most abundant polysaccharide produced by plants, and on the transcriptional network regulating the biosynthesis of secondary walls. His long-term goal is to apply the knowledge learned from study of secondary wall biosynthesis into genetic engineering of the quantity and quality of fibers and wood.

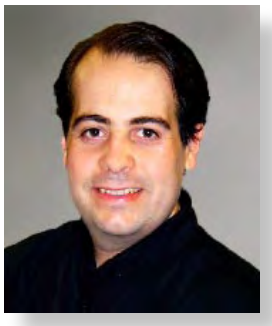
Arthur C. Neish Young Investigator Awards

Reinhard Jetter

Reinhard Jetter received his undergraduate training in chemistry at the University of Munich, Germany, specializing on organic synthesis and the physical chemistry of antiaromatic compounds. He then did his Ph.D. in Botany at the University of Kaiserslautern, Germany, in the laboratory of Markus Riederer (1993), studying the crystallization of plant waxes. Reinhard went on to the Institute of Biological Chemistry at Washington State University, where he worked as a Postdoctoral Fellow with Rod Croteau (1994-96). His work at the IBC focused on the cloning and characterization of enzymes involved in the biosynthesis of conifer resin diterpenoids. Next, he worked as a Research Associate and independent group leader in the Biology Department at the University of Wuerzburg, Germany (1996-2003), where he began his investigations into the polyketides and triterpenoids found in plant skins. Finally, he joined the University of British Columbia in 2003, as Canadian Research Chair in Plant Natural Products Chemistry, with a cross-appointment between the Departments of Botany and Chemistry. He is leading a group of chemists and biologists focusing on the various aspects of plant surfaces, using a wide range of techniques. His current projects are highly interdisciplinary, ranging from molecular genetics over enzyme mechanism and chemical product identification to the physiology of water transport and the chemical ecology of plant-insect-interactions.

Joseph Jez

Joseph M. Jez received his B.S. degree in Biochemistry with Honors and an English minor from Penn State University (1992) and his Ph.D. degree in Biochemistry & Molecular Biophysics from the University of Pennsylvania (1998). His graduate work with Professor Trevor M. Penning focused on understanding the chemical mechanism and the determinants of steroid recognition in mammalian aldo-keto reductases. As an NIH postdoctoral fellow, he joined Professor Joseph P. Noel's group in the Structural Biology Laboratory of the Salk Institute for Biological Studies (1998-2001), where his work involved structural, mechanistic, and protein engineering studies of plant (or type III) polyketide synthases. He was a Scientist at Kosan Biosciences (2001-2002) before joining the faculty of the Donald Danforth Plant Science Center in 2002 as an Assistant Member. He is an Adjunct Assistant Professor of Biology at Washington University in St. Louis and was a recipient of a Presidential Early Career Award for Scientists and Engineers (PECASE) in 2006. Current work in his lab focuses on the biosynthesis of molecules that protect plants from heavy metal toxicity and environmental stresses using approaches that blend biochemistry, plant biology, and protein crystallography. Areas of research interest include sulfur assimilation, protein-protein interactions in cysteine synthesis, redox-regulation of glutathione biosynthesis, and engineering heavy metal tolerance in plants. His lab is also collaborating with Divergence, Inc. (St. Louis, MO) to mechanistically and structurally characterize new protein targets for the development of compounds targeting parasitic nematodes.



Michael Jourdes

Michael Jourdes initially received his B.S. and M.Sc. degree in Organic Chemistry from the University of Poitiers in France, and then his Ph.D. degree in Organic Chemistry in 2003 from the University of Bordeaux. During his Ph.D. studies, he worked under the supervision of Professor Stephane Quideau on polyphenol chemistry. During this time, he particularly focused on the evolution of ellagitannins and formation of flavano-ellagitannins during the aging of red wine in oak barrels. His Ph.D. research was awarded the Best Thesis Award from the French Chemical Society (Aquitaine Section) in 2003 and the First Prize for Doctoral Research form the Amorim Academy in 2004. In 2004, he joined Professor Norman G. Lewis's

group in the Institute of Biological Chemistry at Washington State University as a postdoctoral fellow. His current work focuses on lignin macromolecular configuration and deposition in vascular plants and has led to numberous publications in internationally respected journals.

B. Markus Lange

Bernd Markus Lange is an Assistant Professor at the Institute of Biological Chemistry and the Center for Integrated Biotechnology at Washington State University. He received his Bachelor's and Master's degrees in Chemistry from the University of Bonn and his Doctoral degree in Botany from the University of Munich. Upon graduation, Dr. Lange held postdoctoral positions with Lutz Heide at the University of Tübingen and Rodney Croteau at Washington State University. Subsequently, he led research groups in the biotechnology industry (Novartis Agricultural Research Institute Inc., Torrey Mesa Research Institute of Syngenta and Diversa Inc.). Currently, he holds a position as an Assistant Professor at the Institute of Biological Chemistry (Washington State University). He is also directing the newly formed M.J. Murdock Metabolomics Laboratory, which provides analytical and data analysis services to the university scientific community. His research interests center on using integrative approaches to characterize the regulation of biochemical pathways with particular emphasis on the crosstalk of pathways involved in isoprenoid biosynthesis.

Sarah O'Connor

Sarah O'Connor received a Bachelor of Science degree in Chemistry from the University of Chicago. She received her Ph.D. in Organic Chemistry under the direction of Barbara Imperiali at Caltech and MIT, where her thesis work focused on the synthesis and structural analysis of glycopeptides. Her post-doctoral research in biochemistry was with Chris Walsh at Harvard Medical School, where she studied the biosynthesis of epothilone and several other polyketide and peptide natural products. She began her independent research program at MIT in 2003. Her research group investigates the mechanism and redesign of terpene indole alkaloid biosynthesis.

Dorothea Tholl

Dorothea Tholl received her diploma degree in Biology (1992) and her Ph.D. degree in Pharmaceutical Biology (1996) with Honors from the Technical University of Braunschweig, Germany. Her graduate work with Professor Thomas Hartmann focused on identifying a key regulatory enzymatic step in the biosynthesis of pyrrolizidine alkaloids. Dorothea then worked as a postdoctoral fellow with Rodney Croteau at the Institute of Biological Chemistry, Washington State University from 1996-1997. From 1997-2003, she joined Professor Jonathan Gershenzon's group in the Department of Biochemistry at the Max Planck Institute for Chemical Ecology as a postdoctoral fellow and junior group leader. She next held a joint appointment with the MPI for Chemical Ecology and Eran Pichersky's group at the University of Michigan from 2003-2005. Her work was involved with elucidating the synthesis, cell type-specific regulation and function of volatile terpenes in conifers and Arabidopsis. Since 2005, she has held an Assistant Professor position in the Department of Biological Sciences at Virginia Tech. Her current research interests focus upon the biosynthesis of volatile terpenes in indirect plant defense, the function of volatiles in plant roots, and the activities of volatiles as endogenous stress regulators. The Tholl lab addresses these questions by using an integrated biochemical, molecular and organismal approach.

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2007 PSNA Annual Meeting Program

SATURDAY, JULY 21

4:00-6:00	Executive Committee/Advisory Board Meeting	
6:00-8:00	Reception	

SUNDAY, JULY 22

Session 1: Molecules from Plants: Medicinal, Defense, and Ecological Roles

Chair: Mark Bernards

8:00-8:15	**William H. Danforth**, Chairman, Donald Danforth Plant Science Center Opening Remarks	
8:15-9:00	**Paul Talalay**, Johns Hopkins University School of Medicine PROTECTION AGAINST CANCER: GLUCOSINOLATES AND ISOTHIOCYANATES	S1-1
9:00-9:45	**Birger Lindberg Møller**, University of Copenhagen CYANOGENIC GLUCOSIDES IN PLANTS, INSECTS, AND THE ENVIRONMENT	S1-2
9:45-10:00	Break	
10:00-10:45	**Edgar B. Cahoon**, Donald Danforth Plant Science Center UNDERSTANDING THE BIOSYNTHESIS OF THE TOCOTRIENOL FORM OF VITAMIN E FOR THE BIOFORTIFICATION OF CROPS	S1-3
10:45-11:10	**Massuo J. Kato**, Universidade de São Paulo CHEMICAL SPECIFICITY OF INSECTS BY PIPERACEAE SPECIES	S1-4
11:10-11:40	**Reuben J. Peters**, Iowa State University TO GIBBERELLINS AND BEYOND!	S1-5
11:40-12:00	**Mark A. Berhow**, USDA-ARS NCAUR ANALYSIS OF GLUCOSINOLATES AND PHENOLICS IN BROCCOLI SEEDS AND SPROUTS	S1-6
12:00-1:00	Lunch	

Session 2: Molecules from Plants and Fungi: Medicinal, Defense, and Ecological Roles

Chair: Cecilia McIntosh

1:00-1:45	**A. Douglas Kinghorn**, Ohio State University PLANT SECONDARY METABOLITES AS POTENTIAL THERAPEUTIC AND CHEMOPREVENTIVE AGENTS	S2-1
1:45-2:30	**Jon Clardy**, Harvard University NATURAL PRODUCTS: ONE WAY FORWARD	S2-2
2:30-3:00	**Diter von Wettstein**, Washington State University THE *lys* 3a REGULATORY MUTANT IN BARLEY POINTS THE WAY TO BREAD AND CEREALS FOR CELIAC PATIENTS	S2-3
3:00-3:15	Break	
3:15-4:00	**Andrés Navarrete**, Universidad Nacional Autónoma de México ISOBOLOGRAPHIC ANALYSIS IN THE PLANT-DRUG INTERACTIONS STUDY	S2-4

Session 3: Graduate Student Presentations
Chair: Toni Kutchan

4:00-4:15	**Michael Mazourek**, Cornell University WHERE THERE'S SMOKE, THERE'S FIRE: THE LINK BETWEEN PEPPER AROMA AND PUNGENCY	S3-1
4:15-4:30	**Claudia L. Cardenas**, Washington State University HYDROXYCINNAMOYL COA SHIKIMATE/QUINATE TRANSFERASE: KINETIC STUDIES AND STRUCTURE	S3-2
4:30-4:45	**Lukasz Kutrzeba**, University of Mississippi BIOSYNTHESIS OF SALVINORIN A: INCORPORATION OF STABLE ISOTOPES	S3-3
4:45-5:00	**Ann M. Patten**, Washington State University TARGETED MANIPULATION OF LIGNINS AND UNINTENDED EFFECTS TO THE VASCULAR/STEM STRUCTURE OF PLANTS	S3-4

Poster Summaries 1: Student Posters
Chair: Toni Kutchan

	Metabolic Engineering of Natural Products (Graduate Students)	
5:00-5:05	**Meredith L. Biedrzycki**, Delaware Biotechnology Institute CHEMICAL GENOMICS TO DECIPHER GENES INVOLVED IN ROOT SECRETIONS	PME-1
5:05-5:10	**Jose Condori**, Arkansas State University CLONING AND CHARACTERIZATION OF A NOVEL STILBENE SYNTHASE FROM PEANUT	PME-2
5:10-5:15	**Rigoberto Rios-Estepa**, Washington State University REGULATION OF MONOTERPENOID ESSENTIAL OIL BIOSYNTHESIS IN PEPPERMINT	PME-3
	Herbal Products and Nutraceuticals (Graduate Students)	
5:15-5:20	**Christina M. Coleman**, University of Mississippi ISOLATION AND IDENTIFICATION OF ANTIADHESIVE URINARY METABOLITES FROM CRANBERRY JUICE	PHP-1
5:20-5:25	**Mario Figueroa**, Universidad Nacional Autónoma de México PHYTOTOXIC COMPOUNDS FROM A NEW EMERICELLA SPECIES	PHP-2
5:25-5:30	**Patricia González Barranco**, Universidad Autónoma de Nuevo León IMMUNOMODULATING PROPERTIES OF BIOACTIVE SECONDARY METABOLITES FROM CULTURE BROTH OF TWO MEXICAN STRAINS OF BASIDIOMYCETES	PHP-3
5:30-5:35	**Onyekachi O. Iroanya**, University of Lagos COMPARATIVE ANALYSIS OF THE PHYTOCHEMICAL CONSTITUENTS OF FRESH AND DRY LEAVES OF *GONGRONEMA LATIFOLIA* AND *VERNONIA AMYGDALINA*	PHP-4
5:35-5:40	**Stanimira Krasteva**, University of Vienna FLUORIMETRIC ASSAY OF HISTONE DEACETYLASE INHIBITORS OF PLANT ORIGIN	PHP-5
5:40-5:45	**Juan Francisco Palacios-Espinosa**, Universidad Nacional Autónoma de México ANTINOCICEPTIVE EFFECT OF SALICYLIC ACID DERIVATIVES FROM *BRICKELLIA VERONICIFOLIA*	PHP-6
5:45-5:50	**Araceli Pérez-Vásquez**, Universidad Nacional Autónoma de México PHYTOTOXIC ACTIVITY AND CONFORMATIONAL ANALYSIS OF THYMOL ANALOGS FROM *HOFMEISTERIA SCHAFFNERI*	PHP-9
5:50-5:55	**Ruxandra Popescu**, University of Vienna *RHODODENDRON* SP. EXTRACTS INFLUENCE EUKARYOTIC CELL DIVISION	PHP-11
5:55-6:00	**Isabel Rivero Cruz**, Universidad Nacional Autónoma de México TOXICITY AND ESSENTIAL OIL COMPOSITION OF *POLIOMINTHA LONGIFLORA*	PHP-12

	Natural Products for Pest Management (Graduate Students)	
6:00-6:05	**Martha Vaughan** and **Jung-Hyun Huh**, Virginia Polytechnic Institute FORMATION OF VOLATILE TERPENES IN ROOTS OF *ARABIDOPSIS THALIANA*	PPM-1
	Natural Product Biosynthesis and Biochemistry (Undergraduate Students)	
6:05-6:10	**Josephat Asiago**, East Tennessee State University ISOLATION, ANALYSIS, AND EXPRESSION OF PGT2 AND PGT4, PUTATIVE FLAVONOID GLUCOSYLTRANSFERASE CLONES FROM *CITRUS PARADISI* LEAVES	PNP-1
6:10-6:15	**Amy C. Schroeder**, Donald Danforth Plant Science Center ROLES OF CONSERVED SERINE AND TYROSINE RESIDUES IN THE ACTIVE SITE OF TYROSINE AMMONIA-LYASE: CONTRIBUTIONS TO CATALYSIS, LIGAND BINDING, AND COFACTOR PROCESSING	PNP-2
	Structure Elucidation/Methods for Analysis (Undergraduate Students)	
6:15-6:20	**Philip D. Boes** and **Jamie L. Yost**, Ashland University MEASUREMENT OF THIOPHENE DYNAMICS IN THE MARIGOLD RHIZOSPHERE	PSE-1
6:20-6:25	**David S. Wilcox**, Ashland University DIFFUSIVE SAMPLING METHODS TO MONITOR ALLELOCHEMICAL DYNAMICS	PSE-2
6:30-8:00	Poster Session (all posters)	

MONDAY, JULY 23

Session 4: Alkaloids, Glycoalkaloids, and Paldoxins: Chemistry, Biosynthesis, and Molecular Biology

Chair: Meinhart Zenk

8:00-8:45	**Fumihiko Sato**, Kyoto University BEYOND THE MOLECULAR CHARACTERIZATION OF ISOQUINOLINE ALKALOID BIOSYNTHESIS	S4-1
8:45-9:15	**Sarah E. O'Connor**, Massachusetts Institute of Technology BIOSYNTHESIS OF NEW ALKALOIDS IN PERIWINKLE	S4-2
9:15-10:00	**Birgit Dräger**, Martin Luther University Halle-Wittenberg CALYSTEGINES — SUGAR MIMICS OF TROPANE ALKALOID ORIGIN	S4-3
10:00-10:15	Break	
10:15-11:00	**Kent F. McCue**, USDA POTATO GLYCOALKALOIDS: METABOLIC REGULATION, FOOD QUALITY AND SAFETY	S4-4
11:00-11:45	**M. Soledade C. Pedras**, University of Saskatchewan LEARNING FROM NATURE: PALDOXINS FOR THE TREATMENT OF PLANT DISEASES	S4-5
11:45-12:05	**Supaart Sirikantaramas**, Chiba University ADAPTIVE EVOLUTION OF DNA TOPOISOMERASE I IN CAMPTOTHECIN- PRODUCING PLANTS	S4-6
12:05-1:00	Lunch	

Session 5: Plant Biochemical Pathways, Enzymology, Metabolomics, and Transcriptomics

Chair: David Gang

1:00-1:45	**Kazuki Saito**, Chiba University/RIKEN PHYTOCHEMICAL GENOMICS IN *ARABIDOPSIS* AND TOWARD NON-MODEL PLANTS: INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS	S5-1

1:45-2:30	**Brenda S.J. Winkel**, Virginia Polytechnic Institute and State University FANCY MEETING YOU HERE! A TALE OF MOONLIGHTING IN PLANT METABOLISM	S5-2
2:30-3:00	**Joseph M. Jez**, Donald Danforth Plant Science Center REGULATORY PROTEIN-PROTEIN INTERACTIONS IN PLANT CYSTEINE BIOSYNTHESIS	S5-3
3:00-3:15	Break	
3:15-3:35	**Edward M. Davis**, Washington State University STRUCTURAL DETERMINANTS OF A MODEL MONOTERPENE CYCLASE, (-)-(4S)-LIMONENE SYNTHASE	S5-4
3:35-3:55	**Chang-Jun Liu**, Brookhaven National Laboratory STRUCTURE-FUNCTION INSIGHTS TO PLANT SMALL MOLECULE METHYLTRANSFERASES	S5-5
3:55-4:15	**Karin Springob**, Donald Danforth Plant Science Center NOVEL HEXAKETIDE SYNTHASES PUTATIVELY INVOLVED IN THE BIOSYNTHESIS OF NAPHTHOQUINONES	S5-6
4:15-5:00	**Katrina Cornish**, Yulex Corporation BIOCHEMICAL REGULATION OF RUBBER BIOSYNTHESIS AND A PROFITABLE RUBBER CROP	S5-7

Poster Summaries 2: Student and Postdoctoral Posters

Chair: Toni Kutchan

Natural Product Biosynthesis and Biochemistry (Graduate Students)

5:00-5:05	**Jennifer K. Cooke**, East Tennessee State University ISOLATION AND IDENTIFICATION OF THE PUTATIVE GLUCOSYLTRANSFERASE PGT8 FROM *CITRUS PARADISI*	PNP-6
5:05-5:10	**Maria Diaz-Chavez**, Leibniz Institute of Plant Biochemistry ISOLATION AND CHARACTERIZATION OF TWO CYTOCHROME P450S FROM THE BIOSYNTHESIS OF STYLOPINE	PNP-7
5:10-5:15	**Andreas Gesell**, University of Victoria IDENTIFICATION OF CYP450 719B1 AS SALUTARIDINE SYNTHASE	PNP-8
5:15-5:20	**Nadja Grobe**, Donald Danforth Plant Science Center ENDOGENOUS MORPHINE IN MAMMALS	PNP-9
5:20-5:25	**Upul Hathwaik**, University of Nevada, Reno SCREENING *CHRYSOTHAMNUS NAUSEOSUS* (RABBIT BRUSH) POPULATIONS FOR VARIATION IN RUBBER CONTENT AND QUALITY	PNP-10
5:25-5:30	**Thongchai Koobkokkruad**, Chulalongkorn University INDIVIDUAL CORRELATION OF ARTEMISININ CONTENT IN GAMMA-IRRADIATED PLANTLETS AND THEIR *EX VITRO* PLANTS OF *ARTEMISIA ANNUA*	PNP-11
5:30-5:35	**Eric McDowell**, University of Arizona COMPARATIVE GENOMICS OF TRANSCRIPTIONAL CONTROL OF PLANT SPECIALIZED METABOLISM	PNP-12
5:35-5:40	**Cesar Nopo-Olazabal**, Arkansas State University INDUCTION OF STILBENE FORMATION IN HAIRY ROOTS OF *NICOTIANA BENTHAMIANA*	PNP-13
5:40-5:45	**Turlapati V. Phanikanth**, Washington State University TOWARDS UNDERSTANDING THE PHYSIOLOGICAL ROLES OF LACCASES IN *ARABIDOPSIS THALIANA* AND *TELLIMA GRANDIFLORA*	PNP-14
5:45-5:50	**Daniel G. Vassão**, Washington State University BIOSYNTHESIS OF BIOACTIVE 9,9′-DEOXYGENATED LIGNANS, NORDIHYDROGUAIARETIC ACID AND CONOCARPAN	PNP-15

	Natural Product Biosynthesis and Biochemistry (Postdoctorals)	
5:50-5:55	**Zhenzhan Chang**, Samuel Roberts Noble Foundation STRUCTURAL STUDY OF ALLENE OXIDE SYNTHASE INVOLVED IN JASMONIC ACID BIOSYNTHESIS	PNP-20
5:55-6:00	**Sung-Jin Kim**, Washington State University ALLYLIC DOUBLE BOND REDUCTASES IN *ARABIDOPSIS* AND *PINUS* SPECIES	PNP-21
6:00-6:05	**Sangaralingam Kumaran**, Donald Danforth Plant Science Center REGULATORY PROTEIN-PROTEIN INTERACTIONS IN PLANT CYSTEINE BIOSYNTHESIS	PNP-22
6:05-6:10	**Taiji Nomura**, Kyoto University BIOSYNTHESIS OF DEFENSIVE SECONDARY METABOLITES, BENZOXAZINONES, IN POLYPLOID WHEAT	PNP-23
6:10-6:15	**Marina Varbanova**, Michigan State University A CYTOCHROME P450 ENZYME FROM *ILLICIUM PARVIFLORUM* CAPABLE OF CATALYZING THE FORMATION OF A METHYLENEDIOXY BRIDGE ON EUGENOL TO PRODUCE SAFROLE	PNP-24
6:15-6:20	**Hong Yang**, Washington State University IDENTIFICATION AND CHARACTERIZATION OF AROGENATE DEHYDRATASE(S) IN *ARABIDOPSIS*: COMPARISON TO A BACTERIAL PREPHENATE DEHYDRATASE	PNP-25
	Structure Elucidation Methods for Analysis (Postdoctorals)	
6:20-6:25	**Yasuhiro Higashi**, Donald Danforth Plant Science Center SUCCESSIVE PROCESSING OF *ARABIDOPSIS* SEED PROTEINS REVEALED BY PROTEOMIC ANALYSIS	PSE-3
6:30-8:00	Poster Session (all posters)	

TUESDAY, JULY 24

Session 6: Biodiversity and Plant Biopolymers

Chair: Brenda Winkel

8:00-8:45	**Peter H. Raven**, Missouri Botanical Garden and Washington University, St. Louis BIODIVERSITY AND OUR FUTURE	S6-1
8:45-9:30	**Zheng-Hua Ye**, University of Georgia GENOMIC ANALYSIS OF SECONDARY WALL BIOSYNTHESIS	S6-2
9:30-10:15	**Debra Mohnen**, University of Georgia PECTIN: STRUCTURE, BIOSYNTHESIS AND FUNCTIONS OF A COMPLEX WALL POLYSACCHARIDE	S6-3
10:15-10:30	Break	
10:30-11:00	**Michaël Jourdes**, Washington State University RECENT ADVANCES IN LIGNIN STRUCTURE AND ASSEMBLY: A COMPELLING 21ST CENTURY CHALLENGE	S6-4
11:00-11:30	**David Shintani**, University of Nevada, Reno THE FUNCTIONAL IDENTIFICATION OF RUBBER BIOSYNTHETIC GENES IN PLANTS	S6-5
11:30-12:00	**Reinhard Jetter**, University of British Columbia SECONDARY METABOLITES AT THE PLANT SURFACE: BIOLOGICAL CHEMISTRY OF CUTICULAR TRITERPENOIDS	S6-6
12:00-1:00	Lunch	

Session 7: Protein Biochemistry, Enzymology, and Metabolism
Chair: Reuben Peters

1:00-1:20	**Jinggao Liu**, USDA-ARS STEREOSPECIFIC OXIDATIVE COUPLING OF HEMIGOSSYPOL TO FORM (+)-GOSSYPOL IN MOCO COTTON IS CONTROLLED BY A DIRIGENT PROTEIN	S7-1
1:20-1:40	**Kye-Won Kim**, Washington State University TOWARDS DEFINING THE BIOCHEMICAL MECHANISM OF STEREOSELECTIVE MONOLIGNOL (RADICAL) COUPLING WITH DIRIGENT PROTEINS: A SITE-DIRECTED MUTAGENESIS APPROACH	S7-2
1:40-2:05	**Toshiaki Umezawa**, Kyoto University SUBUNIT COMPOSITION OF HINOKIRESINOL SYNTHASE CONTROLS GEOMETRICAL SELECTIVITY IN HINOKIRESINOL FORMATION	S7-3
2:05-2:25	**Franck E. Dayan**, USDA-ARS NPURU BIOSYNTHESIS OF LIPID RESORCINOLS AND BENZOQUINONES IN ISOLATED SECRETORY ROOT HAIRS OF *S. BICOLOR*	S7-4
2:45-6:00	Visit Missouri Botanical Garden on birthday of founder Henry Shaw (July 14, 1800-August 25,1889)	
7:15-9:30	Banquet and Awards	

WEDNESDAY, JULY 25

Session 8: Enabling Technology and Metabolism
Chair: Franck Dayan

8:00-8:30	**David Gang**, University of Arizona CONTROL OF METABOLISM IN A SINGLE CELL TYPE	S8-1
8:30-9:00	**Oliver Prange**, Carl Zeiss MicroImaging LASER MICRODISSECTION AND PRESSURE CATAPULTING (LMPC): ENABLING TECHNOLOGIES FOR PLANT RESEARCH	S8-2
9:00-9:30	**Mark Lange**, Washington State University DEVELOPMENT AND EXPERIMENTAL TESTING OF KINETIC MATHEMATICAL MODELS FOR ISOPRENOID ESSENTIAL OIL BIOSYNTHESIS IN MINT	S8-3
9:30-10:00	**Dorothea Tholl**, Virginia Polytechnic Institute and State University SYNTHESIS AND FUNCTION OF VOLATILE TERPENES: NEWS FROM THE *ARABIDOPSIS* MODEL	S8-4
10:00-10:15	Break	
10:15-10:35	**Daniel K. Owens**, East Tennessee State University IDENTIFICATION AND CHARACTERIZATION OF PUTATIVE GLUCOSYL TRANSFERASES FROM *CITRUS PARADISI*	S8-5
10:35-10:55	**Fabricio Medina-Bolivar**, Arkansas State University TRICHLOROETHYLENE INDUCES FORMATION OF STILBENOID COMPOUNDS AND ANTIOXIDANT ACTIVITY IN PEANUT ROOTS	S8-6
10:55-11:15	**Ganapathy Sivakumar**, Arkansas State University HPLC-MS SCREENING FOR RESVERATROL AND ITS DERIVATIVES IN HAIRY ROOT CULTURES OF PEANUT	S8-7
11:15-12:30	Business Meeting / Boxed Lunch	
12:30	Meeting adjourns	

Poster Session (not including oral summaries)

	Herbal Products and Nutraceuticals	
	Juan Francisco Palacios-Espinosa, UNAM ANTIHYPERGLYCEMIC EFFECTS OF *BRICKELLIA VERONICIFOLIA* IN STREPTOZOCIN-INDUCED DIABETIC RATS	PHP-7
	Juan Francisco Palacios-Espinosa, UNAM PHYTOTOXIC POTENTIAL OF *BRICKELLIA CAVANILLESII*	PHP-8
	Araceli Pérez-Vásquez, UNAM CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *HOFMEISTERIA SCHAFFNER*I (A. GRAY) R. M. KING & H. ROBINSON (ASTERACEAE)	PHP-10
	Natural Product Biosynthesis and Biochemistry	
Undergrad Students	**Kiani Arkus**, Donald Danforth Plant Science Center STRUCTURE/FUNCTION STUDIES AND PROTEIN ENGINEERING OF ATP-DEPENDENT PEPTIDE LIGASES	PNP-3
	Anna Locke, Donald Danforth Plant Science Center A SURVEY OF SPHINGOLIPIDS IN MEMBERS OF THE VIRIDIPLANTAE	PNP-4
	James Moten, New Mexico State University CORRELATION BETWEEN EXPRESSION OF ACYLTRANSFERASES AND DIVERSITY OF COMPOUNDS CAUSING PUNGENCY IN CAPSICUM VARIETIES	PNP-5
Graduate Students	**Yeon-Bok Kim**, Seoul National University DITERPENE RESIN BIOSYNTHESIS IN PINUS DENSIFLORA IS REGULATED BY TWO OF THE METHYLERYTHRITOL PHOSPHATE PATHWAY GENES, DXS AN	PNP-16
	Francis Mann, Iowa State University CHIMERIC ANALYSIS OF CLASS II DITERPENE SYNTHASES	PNP-17
	Qiang Wang, Iowa State University CLARIFYING RICE GIBBERELLIC ACID METABOLISM	PNP-18
	Ke Zhou, Iowa State University INVESTIGATIONS OF SUBSTRATE AND PRODUCT SPECIFICITY IN CLASS I DITERPENE SYNTHASES	PNP-19
Postdocs	**Sivakumar Swaminathan**, Iowa State University A RICE CYTOCHROME P450 KAURENE OXIDASE HOMOLOG WITH A POTENTIAL ROLE IN LABDANE- RELATED DITERPENOID PHYTOALEXIN BIOSYNTHESIS	PNP-26
General Session	**Mark Bernards**, University of Western Ontario SUBERIN BIOSYNTHESIS: A METABOLOMICS APPROACH	PNP-27
	Wanchai De-Eknamkul, Chulalongkorn University *IN VITRO* PLANT, CALLUS AND ROOT CULTURES OF *PLUMBAGO INDICA* AND THEIR BIOSYNTHETIC POTENTIAL OF PLUMBAGIN	PNP-28
	Frédéric Marsolais, Agriculture and Agri-Food Canada MODULATION OF FREE AMINO ACIDS AND GAMMA-GLUTAMYL DIPEPTIDES IN RESPONSE TO STORAGE PROTEIN DEFICIENCY IN SEEDS OF COMMON BEAN	PNP-29
	Susan McCormick, USDA-ARS NCAUR PHYTOTOXICITY OF TRICHOTHECENES USING AN *ARABIDOPSIS* DETACHED LEAF ASSAY	PNP-30
	Anan Ounaroon, Naresuan University MOLECULAR CLONING OF STRICTOSIDINE SYNTHASE FROM *MITRAGYNA SPECIOSA*	PNP-31
	Woraporn Putalun, Khon Kaen University MULTIPLE SHOOT REGENERATION AND ARTEMISININ PRODUCTION IN *ARTEMISIA ANNUA* L. USING THIDIAZURON	PNP-32

	Sanja Roje, Washington State University METABOLISM OF FLAVIN NUCLEOTIDES IN PLANTS	PNP-33
	Worapan Sitthithaworn, Srinakharinwirot University REGULATORY ROLE OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE IN PLAUNOTOL BIOSYNTHESIS DURING *CROTON STELLATOPILOSUS* LEAF DEVELOPMENT	PNP-34
	Pimpimon Tansakul, Prince of Songkla University *c*DNA CLONING AND EXPRESSION PROFILING OF DXR GENE ENCODING 1-DEOXY-D-XYLULOSE 5-PHOSPHATE REDUCTOISOMERASE FROM *CROTON STELLATOPILOSUS* OHBA	PNP-35
	Juraithip Wungsintaweekul, Prince of Songkla University BIOSYNTHESIS OF *Beta*-SITOSTEROL AND STIGMASTEROL PROCEEDS EXCLUSIVELY VIA MEVALONATE PATHWAY IN THE DISORGANIZED CELL SUSPENSION CULTURES OF *CROTON STELLATOPILOSUS*	PNP-36
	Structure Elucidation/Methods for Analysis	
Postdocs	**Muntha K. Reddy**, University of Mississippi PHENOLIC ACIDS, GALLO-, AND ELLAGITANNINS FROM *PUNICA GRANATUM* L.	PSE-4
General Session	**Filippo Imperato**, Università della Basilicata THREE NEW FLAVONOIDS FROM THE FERN *DRYOPTERIS VILLARII*	PSE-5
	Filippo Imperato, Università della Basilicata APIGENIN 4′ -O-(*p*-COUMAROYL GLUCOSIDE) FROM THE FERN *DRYOPTERIS VILLARII*	PSE-6
	Marcos Soto-Hernández, Colegio de Postgraduados, Montecillo TAXANES OF THE FOLIAGE OF THE MEXICAN YEW, *TAXUS GLOBOSA*	PSE-7
	Natural Products for Pest Management	
General Session	**Jan F. Stevens**, Oregon State University MEADOWFOAM-BASED BIOHERBICIDES	PPM-2

Meeting Abstracts

Session I: Molecules from Plants: Medicinal, Defense, and Ecological roles

S1-1

PROTECTION AGAINST CANCER: GLUCOSINOLATES AND ISOTHIOCYANATES

Paul Talalay and Jed W. Fahey (Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205)

Consumption of diets rich in plants reduces the risk of cancer and chronic degenerative diseases. Cruciferous vegetables (*Brassicaceae*), widely-consumed globally, are especially protective, and their very high concentrations of glucosinolates (GS) are major contributors to protection. More than 120 GS have been isolated from plants. They are aliphatic, aromatic, and indole □-thioglucoside *N*-hydroxysulfates, derived from amino acids by unique reactions leading to at least 10 distinct chemical classes; fully one-quarter originate from methionine and contain sulfur in their side-chains. The effects of GS on animal tissues are due to their enzymatic conversion to isothiocyanates (ITC) by plant □-thioglucosidases (myrosinases), which coexist (but are physically segregated) in plant cells. If plant myrosinase is inactivated, GS are hydrolyzed by the microflora of the mammalian intestinal tract. The efficiency of conversion of GS to ITC by microbial myrosinases varies greatly between individuals, but is relatively constant in each individual over time. This conversion efficiency may control the protective effectiveness of GS. Among these compounds, sulforaphane (SF; $CH_3S(O)$-$(CH_2)_4$-N=C=S) and its GS (glucoraphanin) isolated from broccoli, have attracted widespread interest. SF is the major, very potent, inducer of cytoprotective "phase 2" genes, and inhibits the formation of a number of chemically- and physically-induced, as well as genetically-determined tumors. In addition to its antitumor effects, SF has a broad spectrum of other properties, including its ability to induce cell cycle arrest and apoptosis, inhibit the growth of tumors, suppress inflammation, inhibit angiogenesis, prevent metastasis, and even inhibit the growth of *Helicobacter pylori,* a major cause of stomach cancer. Progress in exploring the broad range of potential therapeutic and protective functions of GS has been hampered by the lack of efficient large scale methods for their isolation. Centrifugal Countercurrent Chromatography has now been developed to attain this goal.

S1-2

CYANOGENIC GLUCOSIDES IN PLANTS, INSECTS AND THE ENVIRONMENT

Birger Lindberg Møller, Thomas Hamann, Mika Zagrobelny, Nanna Bjarnholt, Raquel Sanchez, and Søren Bak (University of Copenhagen, Department of Plant Biology, Plant Biochemistry Laboratory and Center for Molecular Plant Physiology (PlaCe))

Cyanogenic glucosides are widely distributed natural products in the plant kingdom and are found in important crop plants like sorghum, barley, cassava, clover, flax, lotus and almonds. Plants also contain degradative enzymes that upon cellular disruption of the plant tissue are brought in contact with the cyanogenic glucosides causing hydrogen cyanide release. This binary system - two sets of components which separately are chemically inert – provides cyanogenic plants with an immediate chemical defense response to herbivores and pathogens causing tissue damage. The biosynthetic pathway is catalyzed by two membrane bound cytochrome P450s and a soluble UDPG-glucosyltransferase and is highly channelled. The channeling mechanism is studied (collaboration with the Sligar laboratory, Urbana) by molecular modeling and by assembly of the metabolon into synthetic nano-discs possessing the characteristics of a soluble protein. Biochemical knowledge of the pathway has enabled predicted metabolic engineering. The trait of cyanogenesis is about 430 million years old enabling co-evolution of cyanogenic plants and their herbivores and pests. The burnet moth *Zygaena filipendulae* is able to sequester the cyanogenic glucosides present in its host plant *Lotus corniculatus* as well as to *de novo* synthesize the compounds. We have demonstrated that these cyanogenic glucosides play intimate roles in the life cycle of the burnet moth *e.g*. as pheromone attractant, mating determinant, as nuptial gifts and in defense. Cyanogenic glucosides present in plant material used as green manure or left on the ground leaches into surface and ground water in amounts exceeding the EU and WHO approved threshold values for drinking water.

S1-3

UNDERSTANDING THE BIOSYNTHESIS OF THE TOCOTRIENOL FORM OF VITAMIN E FOR THE BIOFORTIFICATION OF CROPS

Edgar B. Cahoon[1], Sarah C. Hunter[2], and Felix R. Solomon[1] ([1]Donald Danforth Plant Science Center and [2]Plant Genetics Research Unit, United States Department of Agriculture-Agricultural Research Service, Donald Danforth Plant Science Center, 975 North Warson Road, Saint Louis, Missouri 63132 USA)

Tocotrienols and tocopherols comprise the vitamin E family of antioxidants in plants. While tocopherols are found in nearly all plant organs, the occurrence of tocotrienols is limited primarily to the seed endosperm of monocots, including important cereal grains such as barley, oats, and rice. Both forms of vitamin E are potent antioxidants that scavenge free radicals and protect polyunsaturated fatty acids from oxidative breakdown. The first step of vitamin E biosynthesis is the condensation of homogentisate and a prenyldiphosphate. We have recently identified a monocot seed enzyme homogentisate geranylgeranyl transferase (HGGT) that catalyzes the initial step of tocotrienol biosynthesis. cDNAs for HGGT from barley, wheat, and rice encode polypeptides that are structurally related, but phylogenetically distinct from homogentisate phytyl transferases (HPT), which catalyze the first step in tocopherol biosynthesis. Transgenic expression of barley HGGT conferred tocotrienol-biosynthetic ability to *Arabidopsis* leaves, indicating that HGGT is the only unique enzyme in the tocotrienol biosynthetic pathway. Recombinant barley HGGT obtained from insect cell expression displayed a five-fold greater activity with geranylgeranyl diphosphate (GGDP) than with phytyl diphosphate (PDP). By contrast, recombinant *Arabidopsis* HPT was 50- to 80-fold more active with PDP than with GGDP. We are currently mapping amino acid residues that confer functional differences between HGGT and HPT. HGGT also has biotechnological potential for increasing the vitamin E antioxidant content of crop plants. For example, the transgenic expression of barley HGGT yielded up to a six-fold increase in the total vitamin E content of corn and soybean seeds and resulted in eight to ten-fold increases in the tocopherol and tocotrienol content of cassava roots and leaves.

S1-4

CHEMICAL SPECIFICITY OF INSECTS BY PIPERACEAE SPECIES

Massuo J. Kato[1], Clécio Souza Ramos[1] and Sergio A. Vanin[2] ([1]Instituto de Química, Universidade de São Paulo, CP 26077, 05513-970 São Paulo, Brazil, [2]Instituto de Biociências, Universidade de São Paulo, CP 26077, 05513-970 São Paulo, Brazil)

The structural diversity of secondary compounds from Piperaceae species include amides, phenylpropanoids, lignan/neolignans, prenylated benzoic acids, chromenes, alkaloids, polyketides and a plethora of mixed biosynthetic pathway compounds. In spite of several of them having insecticidal activities, Lepidoptera, Coleoptera and Hemiptera species feed on *Piper* leaves in a clear pattern. High specificity has been observed between Lepidoptera (*Quadrus u-lucida*, *Heraclides hectorides, H. brasiliensis*) and lignan/neolignan-containing species (*Piper regnellii* and *P. solmsianum*), and Coleoptera (*Naupactus bipes*) and benzoic acid-containing *Piper* species (*P. gaudichaudianum* and *P. aduncum*). The major biotransformation reaction of the tetrahydrofuran lignan grandisin was the demethylation reaction by *Q. u-lucida* and *H. hectorides* to yield di-4,4′ -*O*-demethylgrandisin during the digestive process of leaves of *P. solmsianum*, while in the case of *P. regnellii* the cleavage of propenyl side-chain of dihydrobenzofuran neolignans by *H. brasiliensis* and *Q. u-lucida* to yield the benzaldehyde or benzoic acid derivatives was observed. The caterpillars of *H. brasiliensis* and the beetle *Naupactus bipes* sequestered the dihydrobenzofuran neolignans and benzoic acids from *P. regnellii* and *P. gaudichaudianum*, respectively.

FAPESP, CAPES, and CNPq

S1-5

TO GIBBERELLINS AND BEYOND!

Reuben J. Peters (Department of Biochemistry, Biophysics, & Molecular Biology
Iowa State University, Ames, IA)

The central role of gibberellic acid (GA) phytohormones in plant growth and development, and the utility of GA metabolic mutants in the "Green Revolution", has stimulated intense interest in study of this biosynthetic pathway. A major focus of our research is enzymatic analysis of GA biosynthesis, which is uniquely initiated by the consecutive action of two distinct classes of diterpene synthases/cyclases, *ent*-copalyl diphosphate synthase (CPS) and kaurene synthase (KS), followed by the action of a mechanistically unusual, multiply reactive cytochrome P450, kaurene oxidase (KO). Mechanistic analysis of each of these enzymes from *Arabidopsis* will be presented. In addition, the absolute requirement for GA production in all flowering plants provides a reservoir of biosynthetic genes whose duplication enables derivation of alternative metabolic pathways. This has happened repeatedly throughout plant evolution, as evidenced by the known presence of almost 7,000 such labdane-related diterpenoid natural products, representing ~5% of all known natural products. Rice produces more than twenty such compounds, many of which serve as antibiotics, via a complex biosynthetic network. Building on our biochemical studies of the *Arabidopsis* GA enzymes, another major focus in our group is a functional genomics based approach towards elucidation of the biochemical activity of the expanded families of 4 CPS, 12 KS, and 5 KO genes found in rice. Notably, within each enzymatic family, the various rice genes are quite closely related and form ideal model systems for enzymatic structure-function analysis. Accordingly, we are studying not only the mechanistic basis for catalytic activity, but also the enzymatic determinants underlying the observed substrate and product specificity. The most current results from our studies will be presented, including mechanistic insights into the catalytic mechanism of class II cyclization reactions, evidence for intrasteric regulation of GA biosynthesis, and mechanistic details of the KS cyclization reaction and the multiple reactions catalyzed by KO, as well as an evolutionary switch for diterpene synthase product specificity.

S1-6

ANALYSIS OF GLUCOSINOLATES AND PHENOLICS IN BROCCOLI SEEDS AND SPROUTS

Mark A. Berhow[1], Brent Tisserat[1], Steven F. Vaughn[1], Sandra M. Duval[1] and Gulab N. Jham[2] ([1] USDA, ARS, NCAUR, [2]Universidade Federal de Viçosa (UFV), Brazil)

Plants from the crucifer family (Brassicaceae) have been touted for their health-enhancing effects when incorporated into diets of both humans and animals. Recently, a great deal of research attention has been focused on the glucosinolates, and glucoraphanin specifically, due to the high positive activity of their hydrolysis products in a variety of cancer-related bioassays. However, the seeds and sprouts of the crucifer plants also contain a number of other interesting glucosinolates including glucoiberin and glucoerusin, as well as some other phenolic compounds that may also have key biological activities both for the plant and for the animals that consume them. Compounds isolated from seeds and sprouts from a variety of crucifer-related species including broccoli show that some of these phenolic compounds are present in concentrations similar to those of the glucosinolates, and may have implications in the use of sprouts as a health-supplements.

S2-1

PLANT SECONDARY METABOLITES AS POTENTIAL THERAPEUTIC AND CHEMPREVENTIVE AGENTS

A. Douglas Kinghorn (The Ohio State University)

In the context of cancer, plant secondary metabolites may be seen to be of great interest for not only chemotherapy but also chemoprevention. Six major groups of plant-derived anticancer agents are currently of major interest, namely, derivatives of campotothecin, combretastatin, homoharringtonine, podophyllotoxin, paclitaxel, and the vinca (*Catharanthus*) bisindole alkaloids. In turn, "cancer chemoprevention" has been defined as "a strategy of cancer control by administration of synthetic or natural compounds to reverse or suppress the process of carcinogenesis", and a number of constituents of vegetables, fruits, and spices and other edible plants of quite wide structural variation are of interest for their chemopreventive effects, including carotenoids, curcumin, monoterpenoids, phenolics, and sulforaphane.

Current research in drug discovery from medicinal plants involves a multidisciplinary, collaborative approach, combining botanical, phytochemical, biological, and molecular techniques. Recent progress will be summarized from our laboratory in elucidating potential anticancer agents from tropical plants, including species of the genera *Aglaia* and *Amomum*, as well as potential cancer chemopreventive agents from various common botanical dietary supplements, including noni (*Morinda citrifolia*), mangosteen (*Garcinia mangostana*), and licorice (*Glycyrrhiza glabra*).

(Funding by grant U19 CA52956 from NCI/NIH and from the Ohio State University Comprehensive Cancer Center is acknowledged).

S2-2

NATURAL PRODUCTS: ONE WAY FORWARD

Jon Clardy (Harvard University)

Plants and other organisms make natural products – a hodgepodge of small molecules from many structural classes. In the past, these molecules have informed a great deal of chemistry and biology, although their contributions are not always widely appreciated. Today, we know a lot about what natural products exist, how many of them are biosynthesized, and ways in which some of them can be useful to humans. In spite of their historical contributions and our current understanding, the role of natural products in scientific research and practical applications is diminishing. This talk will emphasize the power of linking genes, small molecules, and biology both for scientific discovery and potential human benefit using recent case studies. The cases involve the search for new molecules from uncultured bacteria (pantocins) and cryptic pathways (bacillaene), chemical signaling of developmental and behavioral switches in the model organism *Caenorhabditis elegans* (dauer formation and alarm), and the chemical ecology of the Southern Pine Beetle, its symbionts, and their predators.

S2-3

THE *lys 3a* REGULATORY MUTANT IN BARLEY POINTS THE WAY TO BREAD AND CEREALS FOR CELIAC PATIENTS

Diter von Wettstein (Department of Crop and Soil Sciences & School of Molecular Biosciences, Washington State University, Pullman WA 99164-6420)

Barley, wheat and rye prolamin grain storage proteins contain peptide stretches that cannot be digested by our stomach, pancreatic and brushborder enzymes and are therefore taken up into the lamina propria of the intestine. There they cause in leucocyte antigen HLA DQ2- or DQ8- positive individuals the autoimmune response erasing the microvillae of the small intestine (2% of the US-population). The promoters of the genes of the B-,C-,□- hordeins and those of the homologous gliadins are methylated in the leaves and are demethylated before transcription can proceed in the developing endosperm. The *lys3a* mutant is unable to carry out this de-methylation. The high-molecular weight hordein D and its homologue HMW glutenin in wheat carry in their promoters a CpG island and are thus not methylated in any tissue including the endosperm. The D-hordein is over-expressed in the *lys 3a* mutant. Cloning of the genes encoding the large two demethylases (Demeter and ROS) identified in barley, wheat and, rice is in progress and will determine if the mutation is in one of these genes. Their structure is known from *Arabidopsis*. Hordein D and HMW glutenin with its resilin like structure is alone responsible for dough elasticity and bread quality: HMW glutenin produced in large quantities in yeast, purified and mixed with flour residue, obtained by washing out from a dough all wheat prolamins, gave excellent elasticity and bread quality (I. Bauer 2005). Since gliadins and B, C, □ hordeins carry most celiac epitopes their elimination by transcriptional silencing is desirable. Their low lysine content makes them also unsuitable for food and feed.

S2-4

ISOBOLOGRAPHIC ANALYSIS IN THE PLANT-DRUG INTERACTIONS STUDY

Andrés Navarrete (Universidad Nacional Autónoma de México)

Herb-drug interaction studies are very limited. There is a dearth of well-documented data in this area and there are few studies that have specifically evaluated herb-drug interactions. The drug interactions can be both pharmacodynamic and pharmacokinetic. Both basic and clinical studies of the pharmacodynamic drug interactions have been performed using isobolographic analysis. This analysis offers a rigorous evaluation of the interactions between two drugs that act together to produce overtly similar effects. The effect of the combination may be a simple addition of the individual effects (*additivity*). In contrast, the effect of the combination can be exaggerated or even attenuated. The exaggerated effect is termed *super-additive* or *synergistic,* whereas the attenuated effect is termed *sub-additive*. This methodology has been applied to the study of the plant-drug interactions. This lecture will describe some studies on the interaction of medicinal plants with activity on CNS (*Variana procera, Ternstroemia pringlei, Casimiroa edulis*), or antiasthmatic properties (*Gnaphalium libmannii, Bougainvillea glabra*), as well as clinically used drugs. The potential uses of this methodology to define the best synergistic combinations of natural products will also be discussed for insecticide, cytotoxicity and other biological activities.

S3-1

WHERE THERE'S SMOKE, THERE'S FIRE: THE LINK BETWEEN PEPPER AROMA AND PUNGENCY

Michael Mazourek[1], David Bolliet[2], Charles Stewart[3], and Molly Jahn[1] ([1]Cornell University, [2]Kalsec Inc., [3]Salk Institute)

Peppers (*Capsicum* sp.) are valued as a spice for their color, pungency and flavor. It is well known that capsaicinoids, a family of alkaloids that accumulate on the placental dissepiment of the fruit, are responsible for the pungency of peppers. However, the nature of the volatiles that contribute to the flavor and aroma of pungent peppers is largely unknown. We recently identified the mutation that results in loss of pungency in bell peppers (*pun1[1]*, *Capsicum annuum*) and another allele of this gene in *Capsicum chinense* (*pun1[2]*) that results in a loss of both pungency and the glandular structures where capsaicinoids accumulate. Habanero peppers (*Capsicum chinense*) are prized as both one of the most pungent and most aromatic peppers. Genetic crosses between habanero and the *pun1[2]* mutant demonstrated pleiotropic effects that link fruit volatiles with capsaicinoid biosynthesis. Headspace SPME-GC-MS and GC-olfactometry identified the tissue within the fruit that synthesizes the volatiles associated with hot pepper aroma as the same tissue that synthesizes capsaicinoids. Principal component analysis of those volatiles in a segregating population demonstrated the dependence of the volatile profile of habaneros on *Pun1*. We believe habanero fruit volatiles may act as an aposematic odor for the non-volatile, painful capsaicinoids, contributing to the peppers' defense against mammalian herbivores. This apparent coevolution will impact the strategies of plant breeders creating new pepper varieties and contribute to our understanding of the factors that underlie the acquisition of new chemical traits that adapt plants to their environments.

S3-2

HYDROXYCINNAMOYL CoA SHIKIMATE/QUINATE TRANSFERASE: KINETIC STUDIES AND STRUCTURE

Claudia L. Cardenas[1], Syed G.A. Moinuddin[1], ChoonSeok Lee[1], Mike Costa[1], Laurence B. Davin[1], ChulHee Kang[2], and Norman G. Lewis[1] ([1]Institute of Biological Chemistry, Washington State University, [2]School of Molecular Biosciences, Washington State University)

Hydroxycinnamoyl CoA: shikimate/quinate hydroxycinnamoyl transferase (HCT) is a member of the acyltransferase family and catalyzes the reversible transfer of acyl groups of hydroxycinnamoyl CoA esters to acceptors, quinic and/or shikimic acids. It is considered to have a central role in the phenylpropanoid pathway, either upstream or downstream of the 3-hydroxylation step. Fully functional recombinant HCT was expressed in *E. coli,* purified, and assayed using as substrates *p*-coumaroyl, caffeoyl, feruloyl, and sinapoyl CoAs, in presence of either shikimic or quinic acids. Determination of K_m and V_{max} values for HCT indicated *circa* 10-fold higher efficiency for *p*-coumaroyl CoA and shikimic acid over the other substrates. Following crystallization and structural analysis of HCT (ternary complex), site-directed mutagenesis was carried out; one of the mutations resulted in abolition of catalytic activity, whereas the two others modified substrate recognition and resulted in lower catalytic activity.

S3-3

BIOSYNTHESIS OF SALVINORIN A: INCORPORATION OF STABLE ISOTOPES

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Salvia divinorum is a hallucinogenic mint endogenous to the Mexican region of Oaxaca. Its bioactive constituent, salvinorin A, is a neoclerodane diterpenoid with selective and high affinity to kappa opioid receptor. We studied the biosynthesis of this compound utilizing several methods of incorporation such as feeding through cuttings and direct stem injection. However, these methods proved to be ineffective in incorporation of isotopically labeled precursors of biosynthetic pathways. We have developed a sterile culture of microshoots, which occurred to be a method of choice for feeding experiments, as well as excellent tool for production of enzymatically active tissues. Incorporation of labeled glucose (1-^{13}C; 2-^{13}C; U-^{13}C), 1-deoxy-D-xylulose (1-^{13}C,3,4-$^{2}H_2$; 1-^{13}C,3-$^{2}H_2$; 1-^{13}C,4-$^{2}H_2$; 1-^{13}C), and L-methionine (Me-^{13}C), provided vast information about the biosynthetic route of salvinorin A. Using ^{13}C-NMR, ^{1}H-NMR and HRMS techniques, we have proved that salvinorin A synthesis in the plant occurs via alternative 2-*C*-methyl-D-erythriol phosphate (MEP) pathway, and that C23 methyl ester comes from *S*-adenosyl-L-methionine dependent methyltransferase. Unambiguous results of these experiments were obtained by peak intensity analysis (1D NMR) with comparison to standards, as well as ^{13}C-^{13}C coupling constants analysis.

S3-4

TARGETED MANIPULATION OF LIGNINS AND PREDICTABLE EFFECTS TO VASCULAR/STEM STRUCTURE/INTEGRITY OF PLANTS

Ann M. Patten, Laurence B. Davin, and Norman G. Lewis (Institute of Biological Chemistry, Washington State University, Pullman, WA)

Lignin removal and/or utilization still represents a major challenge for commercial use of plant tissues whether for biofuels, livestock feeds, lumber or paper manufacture. Mounting evidence indicates that targeted reduction of lignin contents/compositions frequently also result in unintended/adverse effects to plant structure. Such effects are, however, quite predictable given the role that lignins play in fortifying walls of vascular cells in all higher plants. A striking example of the structural variability that can result exists between 2 different plant species altered in *p*-coumarate-3-hydroxylase (*p*C3H) activity: (a) the *Arabidopsis ref8* mutant, which is severely dwarfed and has collapsed xylem, and (b) the *p*C3H-I alfalfa line that has a nearly normal growth habit and stem tensile strength properties. One remarkable difference between both transgenics lies in the early formation of tension wood in alfalfa by *p*C3H-I (relative to wild-type), and this apparently does not occur in *Arabidopsis*. Its formation seems to offset the mechanical weakness that would otherwise accompany its ~64% reduction in lignin contents. Tension wood is, however, generally associated with difficult industrial processing and thus represents a significant challenge to manipulating woody species. As another example, the mutated *Arabidopsis cad-4 cad-5* line results in reduced stem tensile properties, through apparently aborting lignin polymerization at an earlier stage (than wild-type) and forming small amounts of poly-*p*-hydroxycinnamaldehydes instead. These two examples, and others to be described, serve to illustrate the range of adverse side-effects that can occur with alteration of phenylpropanoid metabolism. Careful consideration of metabolic bioengineering *in planta* will be necessary to produce plant lines that will be successful in the field as sustainable resources.

S4-1

BEYOND THE MOLECULAR CHARACTERIZATION OF ISOQUINOLINE ALKALOID BIOSYNTHESIS

Fumihiko Sato (Kyoto University)

Higher plants produce diverse classes of metabolites. Recent progresses in molecular characterization of secondary metabolism offer tremendous potential to improve the production and quality of these chemicals. I report here the possibilities of metabolic engineering in benzylisoquinoline alkaloid biosynthesis. Examples of these include morphine, sanguinarine, and berberine, which are synthesized from tyrosine via reticuline in the Ranunculaceae, Berberidaceae, Papaveraceae, and many other species. The early pathway from tyrosine to reticuline is common among many plant species, whereas there is more diversity in late pathways. Since we have obtained many cDNAs of biosynthetic enzymes in this pathway[1)], we can use several strategies to engineer the yield and quality of benzylisoquinoline alkaloids. 1) The identification of rate-limiting step enzymes (such as 6OMT) and its over-expression have proven to be useful to increase the overall alkaloid yield[2)]. 2) The introduction of a new branch (such as SMT) into the benzophenanthrizine pathway has been shown to be useful to produce novel metabolites[3)] and to dissect metabolic flexibility. 3) The knock-down of a key step (BBE) also showed successful accumulation of a pathway intermediate[4)]. Besides direct metabolic engineering with isolated biosynthetic genes *in vivo*, our current collection of biosynthetic genes provides another possibility to reconstruct the entire biosynthesis in heterologous systems such as *E. coli*. I discuss the potential of this synthetic biological approach as the next generation of metabolite production.

[1)]J Biol Chem **282**:6274-6282 (2007). [2)]Plant Cell Physiol **48**(2):252-62 (2007). [3)]Proc Natl Acad Sci USA **98**(1): 367-372 (2001). [4)]Transgenic Res **16**:363-375 (2007).

S4-2

BIOSYNTHESIS OF NEW ALKALOIDS IN PERIWINKLE

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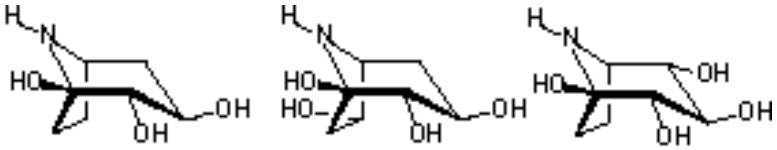
Plant alkaloids are complex natural products and many of these molecules have useful pharmaceutical activities. Despite the importance of plant-derived alkaloid natural products, metabolic engineering efforts in these complex eukaryotic pathways have been somewhat limited. Madagascar periwinkle (*Catharanthus roseus*) produces more than 100 alkaloids from the terpene indole alkaloid family. We describe mechanistic studies and the rational redesign of the central terpene indole alkaloid biosynthetic enzyme. A variety of novel substrate analogs are turned over by the enzyme variants. Moreover, these substrates are processed by later steps of the terpene indole alkaloid pathway resulting in the production of a diverse array of alkaloid derivatives. Our work suggests that integration of enzymes with altered substrate specificity into this complex natural product pathway is a viable strategy for metabolic engineering of novel terpene indole alkaloids.

S4-3

CALYSTEGINES — SUGAR MIMICS OF TROPANE ALKALOID ORIGIN

Birgit Draeger, Stefan Biastoff, Andrea Brock, Nebojsa Jockovic, Heike Kaiser, Anna-Carolin Meier, Ute Richter, Michael Teuber (Martin Luther University Halle-Wittenberg, Institute of Pharmacy, Germany)

Calystegines are non-esterified nortropane alkaloids with three to five hydroxyl groups, initially described from roots of *Calystegia sepium* (hedge bindweed), Convolvulaceae.



Ref: Biastoff, Draeger 2007 CALYSTEGINES in: The Alkaloids, Vol. 64, in press

They are found in all Solanaceae, plants that are renowned for medicinal tropane alkaloids, e.g. atropine and scopolamine, and they occur in many other Solanaceae such as potato or tomato. Calystegines were also identified in Erythroxylaceae, where tropane alkaloids like cocaine are formed, in the Moraceae, and in several Brassicaceae, in which cochlearine is the only tropane alkaloid described so far. Calystegine biosynthesis comprises enzymatic steps that are known from the biogenesis of medicinal tropane alkaloids, e. g. methylation of putrescine and reduction of tropinone. The genes of the corresponding enzymes were cloned from calystegine forming plants, and recombinant proteins/enzymes were expressed. Catalytic characteristics and subcellular localisation of the enzymes in plant tissues are currently being investigated. Calystegines inhibit glycosidases due to their sugar-mimic structure, and they may possess toxic or medicinal potential. In plants, they may act either as rhizosphere attractors for microorganisms and/or as allelopathic agents.

S4-4

POTATO GLYCOALKALOIDS: METABOLIC REGULATION, FOOD QUALITY AND SAFETY

Kent F. McCue[1], Louise V.T. Shepherd[2], Howard V. Davies[2] and William R. Belknap[1] ([1]USDA-Agricultural Research Service and [2]Scottish Crop Research Institute)

Steroidal glycoalkaloids (SGAs) are undesirable secondary metabolites produced in solanaceous plants, including potato, tomato and eggplant. Two tri-glycosylated alkaloids, α-chaconine and α-solanine, occur naturally in potato tubers. The levels of SGA accumulation in tubers are determined by both environmental conditions and genetic background. High levels of SGAs present potential food safety issues, including gastrointestinal distress. Practically, however, tubers with high levels of these compounds are rejected by consumers due to their intense bitter taste. Acute reactions are not encountered at levels below the currently established guideline for new cultivars of 20 mg/100g FW. However, higher levels can occur during cultivation and remain a persistent problem in breeding programs. We have identified and characterized three members of the SGA glycosyl transferasese gene family, namely the genes encoding the UDP-galactose: solanidine galactosyltransferase *Sgt1*, the UDP-glucose:solanidine glucosyltransferase *Sgt2*, and the UDP-rhamnose: β-solanine/ β-chaconine rhamnosyl-transferase *Sgt3*. Reverse genetic manipulation of the *Sgt* gene family using transgenic lines expressing active antisense gene constructs results in a shift of SGA pathway flux, characterized with compensation by other pathway intermediates and products. New *Sgt* gene family members are also being studied to verify their substrate specificity and biosynthetic sequence. Additional studies are underway to use the members of this gene family to completely control accumulation of SGAs in tubers.

S4-5

LEARNING FROM NATURE: PALDOXINS FOR THE TREATMENT OF PLANT DISEASES

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The interaction of plants with their fungal pathogens involves a variety of defense and counter-attack mechanisms operating in both organisms. In many of these interactions, successful fungal invasion leading to eventual plant death includes the production of fungal enzymes involved in the detoxification of phytoalexins (plant chemical defenses produced *de novo* in response to stress). Paldoxins are inhibitors of phytoalexin detoxification conceived to be non-toxic to living systems. To design inhibitors of phytoalexin detoxifying enzymes, i.e. paldoxins, we have been investigating the effects of various crucifer phytoalexins and their structural analogues on fungal detoxification reactions. The fungal enzyme involved in the detoxification of the phytoalexin brassinin, brassinin oxidase, has been purified and was used to screen a variety of brassinin analogues. These studies have revealed structural requirements of brassinin oxidase substrates and paldoxins, as well as mechanisms of detoxification. The most recent results on the analyses of metabolic pathways involved in the interaction between crucifers and their pathogens will be presented. Strategies to prevent and treat plant microbial diseases based on these studies will be discussed.

S4-6

ADAPTIVE EVOLUTION OF DNA TOPOISOMERASE I IN CAMPTOTHECIN- PRODUCING PLANTS

Supaart Sirikantaramas[1], Mami Yamazaki[1] and Kazuki Saito[1,2] ([1]Graduate School of Pharmaceutical Sciences, Chiba University, Japan, and [2]RIKEN Plant Science Center, Japan)

Camptothecin, an anticancer monoterpene indole alkaloid, is produced in many distantly related plant families including *Camptotheca acuminata*, *Nothapodytes foetida* and *Ophiorrhiza pumila*. These plants show resistance to camptothecin, which can induce eukaryotic DNA topoisomease I-mediated DNA damage. Because of its toxicity to a house-keeping enzyme of cells, we raised the interesting question of what mechanism underlies camptothecin detoxification in these plants. Our previous study demonstrated that a transporter is not involved in its detoxification mechanism of *O. pumila*. Therefore, we attempted to clone and characterize topoisomerase I of these plants. A camptothecin sensitivity assay in yeast revealed that topoisomerase I from camptothecin-producing plants is resistant to camptothecin. In contrast, topoisomerase I from camptothecin non-producing plants, including *O. japonica* which is closely related to *O. pumila*, is sensitive to camptothecin. Homology modeling based on the human topoisomerase I structure identified several amino acid substitutions at the residues that affect enzyme structure or contribute to camptothecin binding. Accordingly, to confirm whether those substitutions confer camptothecin resistance, topoisomerase I from *O. japonica* was modified by site-directed mutagenesis creating mutants encoding one substitution. As a result, we identified amino acid substitutions that confer the self-resistance mechanism of camptothecin-producing plants. Our findings provide the evidence of adaptive evolution of topoisomerase I towards camptothecin resistance in camptothecin-producing plant and the effect of specific secondary metabolite on evolution of plants.

S5-1

PHYTOCHEMICAL GENOMICS IN *ARABIDOPSIS* AND TOWARD NON-MODEL PLANTS: INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS

Kazuki Saito (Graduate School of Pharmaceutical Sciences,Chiba University/RIKEN Plant Science Center, Japan)

The completion of the whole genome sequence of *Arabidopsis thaliana* has made it possible to discover the genes involved in metabolism in a high throughput manner by determining gene-to-metabolite correlation through the comprehensive analysis of metabolite accumulation and gene expression. *In silico* co-expression analysis of genes involved in flavonoid metabolism in *Arabidopsis* was performed using a publicly available transcriptome database of DNA microarrays. We inferred a co-expression framework model of the genes involved in the pathways of flavonoids, suggesting specific functions and co-regulation of the genes of pathway enzymes and transcription factors. Changes in flavonoid profiles of wild-type plants and T-DNA insertion mutants of the delimited genes led to the confirmation of gene function (J Biol Chem, doi:10.1074/jbc.M611498200 (2007)). We also applied this strategy to the glucosinolate biosynthetic pathway for identification of MYB transcription factors crucial for aliphatic glucosinolate production (PNAS 104:6478 (2007)). These results suggest that the functional genomics approach by integration of metabolome with transcriptome co-expression analysis provides an efficient way of identifying novel gene functions involved in plant secondary metabolism. This strategy is principally applicable to decipher the function of genes not only for a model plant *A. thaliana* but also for unexplored plants rich with a variety of phychemicals, such as *Ophiorrhiza pumila* root cultures producing the anti-cancer alkaloid camptothecin.

S5-2

FANCY MEETING *YOU* HERE! A TALE OF MOONLIGHTING IN PLANT METABOLISM

Brenda S.J. Winkel, Melissa V. Ramirez, Kevin C. Crosby, Peter A. Bowerman, Jonathan Watkinson, and David E. Saslowsky (Virginia Tech, Blacksburg, VA 24061)

Flavonoids are a well-known class of plant metabolites that play key roles in plant growth and survival and that are also of growing interest as phytonutrients and pharmaceuticals. However, engineering flavonoid biosynthesis has not always been straightforward, as evidenced by the elusive blue rose and efforts to alter flavonoid profiles in edible plants such as tomato. Despite more than a century of work on the biochemistry and physiology of this system, it continues to offer up new surprises and it is clear that much remains to be learned about flavonoid biosynthesis, and cellular metabolism in general.

We recently reported that the first two enzymes of the flavonoid pathway, chalcone synthase (CHS) and chalcone isomerase (CHI), reside not only at the cytoplasmic face of the ER, but also in the nuclei of *Arabidopsis* plants. Analysis of the physical interactions of these two enzymes, together with studies of the determinants of their dual localization, is providing evidence for a previously-unsuspected function for these proteins. Preliminary data from the analysis of CHS-GFP constructs in transgenic plants suggest that CHS may function to activate its own transcription, and thus conceivably the expression of other genes, as well. The data also suggest that CHI could act as a negative regulator of transcriptional activation by binding to CHS at a site that overlaps a putative nuclear localization signal. This new model has prompted the reinterpretation of the effects of flavonoid mutants on gene and protein expression in the context of a “moonlighting” function for flavonoid enzymes in the nucleus, an increasingly common theme for enzymes in plants and other organisms.

S5-3

REGULATORY PROTEIN-PROTEIN INTERACTIONS IN PLANT CYSTEINE BIOSYNTHESIS

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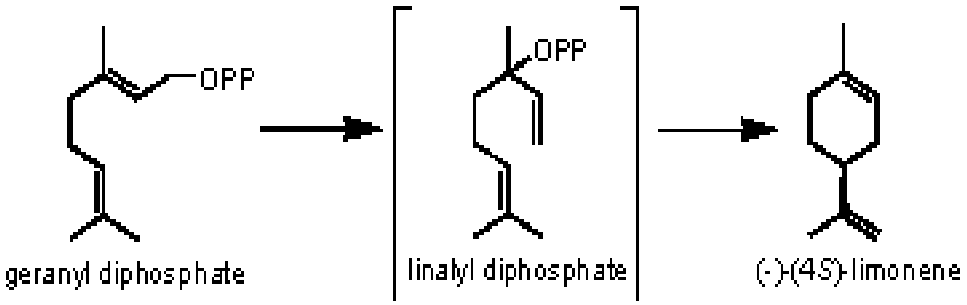
Multienzyme complexes provide cells with a means of organizing molecular networks and regulating metabolic pathways. In plant sulfur metabolism, cysteine biosynthesis is the metabolic link between sulfur assimilation and the myriad of sulfur-containing molecules in the cell. A key regulatory feature of this pathway is the association of the two cysteine biosynthetic enzymes (O-acetylserine sulfhydrylase, OASS, and serine acetyltransferase, SAT) into the cysteine synthase complex. This multienzyme assembly acts as a sensor in a regulatory circuit that coordinates sulfur assimilation and cysteine production. Although the cysteine synthase complex was one of the first macromolecular assemblies to be isolated, the details of how OASS and SAT associate are only beginning to be understood. Our goal is to understand the structural and energetic basis for complex formation and how protein-protein interactions regulate cysteine synthesis in plants. We determined the crystal structure of *Arabidopsis* OASS bound with a peptide consisting of the ten residues found at the C-terminus of *Arabidopsis* SAT (C10 peptide). Interactions with key active site residues lock the peptide in the binding site to block access to the catalytic site of OASS, which explains how its activity is down-regulated upon formation of the cysteine synthase complex. Thermodynamic analysis of complex formation between OASS and the C10 peptide suggests that the C-terminus of SAT provides the major contribution to total binding energy during complex formation. These results provide new insights into the molecular mechanism underlying formation of the cysteine synthase complex and provide a structural basis for the biochemical regulation of cysteine biosynthesis in plants.

S5-4

NEW STRUCTURAL DETERMINANTS OF A MODEL MONOTERPENE CYCLASE, (-)-(4*S*)-LIMONENE SYNTHASE

Edward M. Davis and Rodney Croteau (Institute of Biological Chemistry, Washington State University, Pullman, Washington)

Terpenes are a diverse group of natural products with over 40,000 representative structures. Much of this diversity results from the initial scaffold generated by the cyclization of the prenyl diphosphate precursers, geranyl diphosphate (GPP, C10), farnesyl diphosphate (FPP, C15) and geranylgeranyl diphosphate (GGPP, C20) by a class of enzymes coined the terpene cyclases. (-)-(4*S*)-Limonene synthase (LS) was chosen as a model for this terpene cyclase-mediated reaction, due to its substrate specificity (accepts GPP only), product specificity (94% limonene), and stereochemical fidelity (>99% (-)-antipode). Several site-directed mutations of active-site residues that influence substrate binding, substrate specificity, product outcome and product stereochemistry will be discussed in the context of the recently defined crystal structure.



S5-5

STRUCTURE-FUNCTION INSIGHTS INTO PLANT SMALL MOLECULE METHYLTRANSFERASES

Chang-Jun Liu, Cheng Wang, Xiao-Hong Yu (Biology Department, Brookhaven National Laboratory, Upton, NY 11973)

S-adenosyl-L-methionine (SAM)-dependent methyltransferases are involved in the biosynthesis of a variety of plant small molecule compounds, such as phenylpropanoid derivatives (lignin, flavonoids and isoflavonoids), alkaloids, coumarins, orcinols, and polyalcohols (e.g. inositol). The methylation is essential in determining specific physiological function of the resultant molecule. In recent years, groups of plant type I methyltransferases have been characterized from model plant species such as *Catharanthus roseus* (Madagascar periwinkle) and *Medicago truncatula.* As part of our efforts in exploring the structure-function relationships of those plant small molecule methyltransferases by comparative structural analysis, we determined a set of crystal structure complexes for one of *C. roseus* methyltransferases that is closely related to caffeic acid 3-OMT but specifically functions for methylation of sulfhydryl group of a range of small molecules; and for one isoflavonoid *O*-methyltransferase homologue, IOMT3 from *M. truncatula*, in complex with different substrates. Unique structural features of both crystallized methyltransferases were observed. Of particular interest, besides the active site binding pocket, we identified two additional binding motifs on the surface of the IOMT3 crystals that specifically accommodate either the co-crystallized isoflavone substrates or the methylated products. The surface binding motifs are largely conserved among a group of isoflavonoid *O*-methyltransferases, as well as a few related flavonoid OMT members. The retaining surface binding structural segments is critical for enzyme activities. The underlying molecular mechanisms will be discussed.

S5-6

NOVEL HEXAKETIDE SYNTHASES PUTATIVELY INVOLVED IN THE BIOSYNTHESIS OF NAPHTHOQUINONES

Karin Springob[1,2], Aphacha Jindaprasert[2,3,4], Jürgen Schmidt[2], Wanchai De-Eknamkul[4] and Toni M. Kutchan[1,2] ([1]Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA, [2]Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, 06120 Halle (Saale), Germany, [3]Faculty of Agroindustry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand, [4]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand)

Drosophyllum lusitanicum Link, the dewy pine, and *Plumbago indica* L., the rose-colored leadwort, produce naphthoquinones derived from six acetate units. In order to identify the enzyme that catalyzes the first step in the biosynthesis of these metabolites, two polyketide synthase (PKS) cDNAs were isolated from callus cultures of *D. lusitanicum* and roots of *P. indica*. The translated polypeptides shared 47-69 % identical residues with PKSs from other plant species. Recombinant *D. lusitanicum* PKS and *P. indica* PKS expressed in *Escherichia coli* accepted acetyl-coenzyme A (CoA) as starter and carried out sequential decarboxylative condensations with malonyl-CoA yielding □-pyrones from three to six acetate units. In addition, the recombinant PKSs accepted a variety of aliphatic and aromatic starter CoA esters and produced □-pyrones of various lengths. Naphthoquinones or naphthalenes, however, were not isolated as enzymatic products. Since the longest □-pyrones produced by the recombinant PKSs are hexaketides, and the naphthoquinones in *D. lusitanicum* and *P. indica* are composed of six acetate units, we propose that the novel hexaketide synthases described herein provide the backbone of naphthoquinones.

Session 5: Plant Biochemical Pathways, Enzymology, Metabolomics, and Transcriptomics

S5-7

NATURAL RUBBER BIOSYNTHESIS AND A PROFITABLE GUAYULE CROP

Katrina Cornish[1] and Colleen M. McMahan[2] ([1]Yulex Corporation, Carlsbad, CA 92008, [2]USDA-ARS Western Region Research Center, Albany, CA 94710)

Natural rubber (*cis*-1,4-polyisoprene) is a biopolymer apparently synthesized in plants by a membrane-bound rubber transferase protein complex. The kinetic features of rubber transferases make them a distinct class of *cis*-prenyl transferases. The structure of the particular allylic pyrophosphate initiator used to begin a new rubber polymer and the concentration of the initiator, monomer and divalent cation cofactor, all directly impact biosynthetic rate and final molecular weight and so directly affect both yield and quality. Guayule is a native rubber producing plant that produces high molecular weight rubber in rubber particles that contain very little extraneous protein. These rubber particles are now produced on a commercial scale as latex for the high-end medical and consumer products markets. However, processing large acreages of guayule shrub for latex production leaves around 90% of the biomass as a resin-rich crop residue. For cost-effective, large-scale and sustainable agriculture, 100% utilization of the entire crop is required. Guayule bagasse from the latex extraction process contains about 10% terpenoid resin which may be separately extracted and sold, or left in the bagasse as an added value. However, as the entire cost of producing the finely-ground, hard-wood, lignocellulosic bagasse is born by the value of the latex, the feed-stock cost is essentially nil, and the cost analyses for resin extraction and for conversion to ethanolic biofuels are very favorable, especially in comparison with other lignocellolosic feedstocks such as rice straw, switch grass and corn stover.

Session 6: Biodiversity and Plant Biopolymers

S6-1

BIODIVERSITY AND OUR FUTURE

Peter H. Raven (Missouri Botanical Garden and Washington University, St. Louis)

We have given names to an estimated 1.8 million species of eukaryotic organisms (those other than bacteria and viruses) out of a total of at least 12 million estimated to exist globally. For most of them, we know little or nothing other than some details of morphology and one or more places where they occur. For the other species, five-sixth of the estimated total, we are for the most part unaware of their existence. By considering relatively well-known groups both in the fossil record and at present, we can calculate that the rate of extinction is running at thousands of species per year, most of them unknown, a number that compares unfavorably with the dozen or so species per year estimated to have become extinct over the past 65 million years and one that is rising rapidly. The chief causes of extinction are habitat destruction, global warming, alien invasive species, and hunting and gathering. We depend completely on other organisms for our continued existence on earth, including such factors as maintaining the composition of the atmosphere, protecting topsoil, determining local climates, and regulating the runoff of surface waters. All of our food comes from plants directly or indirectly, and the majority of our medicines worldwide come from, or originally came from, organisms. In the early decades of a revolution in molecular knowledge and of our ability to apply the property of organisms to the promotion of worldwide sustainability, it is a tragedy to be facing the loss of well over half of all species, most of them unknown, by the end of the century we have just entered. What can we do to slow down or reverse this highly unfavorable outcome?

S6-2

GENOMIC ANALYSIS OF SECONDARY WALL BIOSYNTHESIS

Zheng-Hua Ye (Department of Plant Biology, University of Georgia, Athens, GA 30602)

Secondary walls are the major constituent of wood, which is the most abundant form of biomass produced by plants. Secondary walls are composed mainly of cellulose, lignin and hemicellulose. To make secondary walls, genes involved in the biosynthetic pathways of cellulose, lignin and hemicellulose need to be coordinately switched on. Understanding the molecular switches controlling secondary wall biosynthesis in wood is of importance in basic plant biology as well as for potential genetic engineering of wood quality and quantity in tree species. We have been using fibers in *Arabidopsis* as a model to study the molecular mechanisms underlying secondary wall biosynthesis. We have analyzed at the genome level the genes that are activated during secondary wall biosynthesis in fibers. We uncovered key roles of several transcription factors in regulating secondary wall biosynthesis. One of these transcription factors, SND1, together with its homolog were found to be master switches activating the biosynthetic pathways of the secondary wall components (Plant Cell, 18, 3158-3170, 2006; Planta, 225, 1603-1611, 2007). In addition, we discovered a number of additional players in the SND1-mediated transcriptional regulation of secondary wall biosynthesis. We hypothesize that a transcriptional network is involved in the activation of secondary wall biosynthetic genes during wood formation. Further studies of the transcriptional network regulating secondary wall biosynthesis will likely enable us to genetically alter the biosynthetic pathways of individual secondary wall components. Moreover, the knowledge gained from such studies promises to lead to better strategies for genetic manipulation of fiber or wood quality and quantity.

(This work is supported by a grant from the U.S. Department of Energy-Bioscience Division).

S6-3

PECTIN: STRUCTURE, BIOSYNTHESIS AND FUNCTIONS OF A COMPLEX WALL POLYSACCHARIDE

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Pectin is a complex family of polysaccharides with diverse functions in plant defense, growth, development, wall structure, cell-cell adhesion and cell signaling. It also has positive effects on human health and is a gelling and stabilizing agent in the food industry. Pectin comprises 10-30% of plant primary walls and 2-5% of woody tissue constituting biomass of use for biofuel and fine chemical production. Pectic polysaccharides contain 1,4-linked α-D-galacturonic acid (GalA) and consist of homogalacturonan (HG), rhamnogalacturonan I (RG-I) and RG-II. *Arabidopsis* GAUT1 is a Golgi-localized α-1,4-galacturonosyltransferase (GalAT) that catalyzes the transfer of GalA from UDP-GalA onto HG. GAUT1 is part of a 25 member gene superfamily, the GAUT1-related gene family, which consists of three evolutionarily-related clades of 15 GAUT genes and 10 GAUT-like (GATL) genes. Our recent biochemical studies show that GAUT1 associates with at least one other GAUT in protein complexes. Cell wall analyses of multiple GAUT and GATL mutants suggest that distinct family members function in the synthesis of diverse wall polymers, including both pectin and xylan. Based on the function of GAUT1, the high sequence similarity across the gene family, available cell wall composition data from diverse family mutants, and knowledge of fine scale wall polymer structure, we propose that the GAUT1-related gene family encodes GalATs involved in pectin, xylan and other wall polymer synthesis. Research funded by NRI, CSREES, USDA Awards 2003-35318-15377 & 2006-35318-17301 and NSF MCB0313509 and MCB0646109.

S6-4

RECENT ADVANCES IN LIGNIN STRUCTURE AND ASSEMBLY: A COMPELLING 21st CENTURY CHALLENGE

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Lignin biopolymeric structure/mode of assembly have been subjects of unresolved debate for nearly five decades. Until recently, approaches were never designed to distinguish whether they were randomly or non-randomly assembled, or if lignin primary structure could be determined. Some researchers still favor random radical-radical coupling leading to astronomical numbers of isomers (e.g. ~10^{66} per 100 mer unit); our data favor directed radical-radical coupling with explicit template control of lignin primary structure/subsequent template replication. Thus, our progress made in determining lignin primary structure, and the approaches used, are described.

To begin to probe lignin primary structures, new synthetic procedures were developed, as well as improving existing lignin chemical degradative analyses. Various synthetic strategies were also designed to afford model compounds representing each inter-unit linkage cleavage reaction. Determination of frequencies in the main lignin inter-unit linkage (such as 8–*O*–4′ , 8–1′ , 8–5′ , 5–5′ , 4–*O*–5′ and 8–8′) in various transgenic plant lines harboring different lignin contents/composition was carried out; sequential degradative analysis procedures were also applied to various genetically modified plant lines (alfalfa and *Arabidopsis*) of vastly different lignin monomeric compositions/ contents. These analyses demonstrated that frequencies of the main 8–*O*–4′ and 8–1′ linkages were fully proportional to lignin content, but independent of methoxyl group substitution pattern, and other linkage types generally followed suit. The data from these, and the related degradative studies of other inter-unit linkages, are discussed in terms of limited substrate degeneracy during lignin template polymerization, with the chemistry associated varying slightly accordingly to substrate.

S6-5

THE FUNCTIONAL IDENTIFICATION OF RUBBER BIOSYNTHETIC GENES IN PLANTS

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Natural rubber (*cis*-1,4-polyisoprene) is an essential plant-derived raw material required for the manufacture of numerous industrial and medical related products. This elastic polymer is synthesized and sequestered within cytosolic vesicles known as rubber particles. When provided with farnesyl-pyrophosphate (FPP) and isopentenyl-pyrophosphate (IPP), rubber particles alone are sufficient for rubber biosynthesis, indicating that these vesicles must possess the required protein machinery for rubber production. As classical biochemical approaches have thus far failed to identify rubber biosynthetic proteins or genes, we have taken an alternative proteomic- and genomic-based approach with four rubber producing plant species including *Hevea brasiliensis*, *Parthenium argentatum*, *Taraxacum kok-saghyz*, and *Ficus elastica*. From these studies, we have identified a common set of rubber particle-associated proteins and a subset of candidate rubber biosynthetic proteins. Through reverse genetic analyses in *Taraxacum kok-saghyz*, we have functionally identified a number of candidate genes whose altered expression appears to affect rubber yields and/or polymer length. We will discuss the potential of these genes for the improvement of current rubber crops and the development of domestic rubber crop species.

S6-6

SECONDARY METABOLITES AT THE PLANT SURFACE: BIOLOGICAL CHEMISTRY OF CUTICULAR TRITERPENOIDS

Reinhard Jetter (University of British Columbia)

Epidermal plant surfaces are covered by a cuticle, *i.e.* an extracellular membrane consisting of the insoluble polyester cutin and soluble cuticular waxes. The intracuticular portion of the waxes, embedded within the cutin matrix, serves in the primary function to prevent non-stomatal water loss. Our recent results show that triterpenoids, present in the intracuticular wax of a number of plant species, do not contribute to the cuticular transpiration barrier. Another part of the waxes, the epicuticular wax layer, is deposited on the outside of the cutin matrix and is therefore exposed at the very surface of the plant. This epicuticular wax layer is of prime importance for interactions with microbial pathogens and insect herbivores. For example, stem surfaces of diverse Euphorb species are covered with epicuticular wax crystals that reduce the adhesive force of insect feet and hence limit insect mobility on the plant surface, representing a mechanical barrier to walking insects. We have monitored the composition of the intracuticular and epicuticular wax layers over the course of stem development, and found that the appearance of epicuticular crystals is directly correlated with the accumulation of pentacyclic triterpenoids at the plant surface. The same crystal shapes can be reconstituted by in-vitro recrystallization of pure triterpenoids. We conclude that, depending on the plant species and organ, the crystals are formed by β-amyrin, lupeol or epitaraxerol. These epicuticular triterpenoids thus serve important ecological functions. The triterpenoid structures are biosynthesized in complex, highly stereoselective, cyclization reactions catalyzed by single oxidosqualene cyclases (OSCs). We have cloned and characterized several OSCs from *Ricinus communis* and other plant species, and have shown that they are involved in the biosynthesis of cuticular triterpenoids.

S7-1

STEREOSPECIFIC OXIDATIVE COUPLING OF HEMIGOSSYPOL TO FORM (+)-GOSSYPOL IN MOCO COTTON IS CONTROLLED BY A DIRIGENT PROTEIN

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Gossypol is biosynthesized by the free radical coupling of two molecules of hemigossypol to yield two optically active enantiomers, (+)-gossypol and (-)-gossypol. Most commercial cottonseeds have a (+) to (-)-gossypol ratio of about 3:2. However, this ratio can be as high as 98:2 in moco cotton. Cell free extracts of moco cotton tissue preferentially converted hemigossypol to (+)-gossypol (74% (+)-gossypol vs. 26% (-)-gossypol). Addition of hydrogen peroxide in the assay produced four fold more gossypol and also increased the (+)-gossypol percentage to 79%. Adding exogenous oxidases like peroxidase or laccase increased gossypol yield but did not decrease the (+)-gossypol percentage. Exogenous oxidases themselves produce a racemic mixture of gossypol from hemigossypol. These results are consistent with the involvement of a dirigent protein in the stereospecific coupling of hemigossypol for the formation of (+)-gossypol and are inconsistent with the involvement of a stereospecific oxidase. The peroxidase activity of the crude extract is mainly responsible for the generation of hemigossypol free radicals in cotton. Partial purification of the crude moco cotton extract yielded a protein preparation that does not possess any catalytic ability to generate the free radicals and is unable to convert hemigossypol to gossypol but nevertheless guided the stereospecific coupling of hemigossypol free radicals generated by a cotton peroxidase(s) for (+)-gossypol formation in moco cotton. This partially purified (+)-gossypol-forming dirigent protein had two native molecular weights of 77.2 and 122.5 kDa probably corresponding to a monomer and dimer, respectively.

S7-2

TOWARDS DEFINING THE BIOCHEMICAL MECHANISM OF STEREOSELECTIVE MONOLIGNOL (RADICAL) COUPLING WITH DIRIGENT PROTEINS: A SITE-DIRECTED MUTAGENESIS APPROACH

Kye-Won Kim, Damian Guerra, Laurence Davin and Norman Lewis (Institute of Biological Chemistry, Washington State University, Pullman, WA 99164)

The (+)-pinoresinol-forming dirigent protein, DP (Latin: *dirigere*, to guide or to align), facilitates stereoselective coupling of two *E*-coniferyl alcohol (radicals) in the presence of an one-electron oxidase/oxidant. The purpose of this investigation was to begin to establish the nature of key amino acid residues in the monolignol (radical) substrate binding pocket of each DP monomer in the DP protein. Homology comparison of two *Forsythia intermedia* (Dir_Fi) and *Schisandra chinensis* DPs (Dir_Sc) led to identification of twenty-two conserved amino acid residues, particularly phenylalanine (F), isoleucine (I) and serine (S). Site-directed mutagenesis of the Dir_Sc was next carried out for each of these residues, with these being individually replaced by Ala. Following individual expression of each mutated protein in S2 *Drosophila* insect cell cultures, the resulting recombinant proteins were purified to near homogeneity. The effects of mutating each of these positions, as well as possible N-glycosylation sites, on stereoselectivity will be described.

S7-3

SUBUNIT COMPOSITION OF HINOKIRESINOL SYNTHASE CONTROLS GEOMETRICAL SELECTIVITY IN HINOKIRESINOL FORMATION

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Norlignans are one of the major classes of plant phenylpropanoid compounds whose biosynthesis has been receiving widespread interest for heartwood formation, plant defense, and pharmaceutical applications. *Asparagus officinalis* has been known to produce a norlignan, (*Z*)-hinokiresinol, as a phytoalexin and is a good model for studies of norlignan biosynthesis. We purified from the plant a novel enzyme, hinokiresinol synthase (HRS) that converted 4-coumaryl 4-coumarate to (*Z*)-hinokiresinol. Then, we cloned two cDNAs encoding the enzyme. Surprisingly, however, each recombinant HRS (HRSα or HRSβ) expressed in *E. coli* solely converted 4-coumaryl 4-coumarate to unnatural (*E*)-hinokiresinol. By contrast, a mixture of the two recombinant HRSs catalyzed the formation of (*Z*)-hinokiresinol, like the plant protein, indicating that HRSα and HRSβ are two subunits of HRS and that the subunit composition can control the geometrical isomerism of the products. Our findings provide a new insight into the diversity of natural product biosynthesis.

S7-4

APPLICATION OF RNAi TECHNOLOGY TO STUDY LIGNAN BIOSYNTHESIS IN FLAX SPECIES

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Lignans are dimers of phenylpropanoid units. Due to their biological activities, numerous lignans are used for human health. Therefore, their biosynthesis is of high interest. Nevertheless, most pathways leading to different lignan structures are not unravelled up to now. We use different molecular approaches to find candidate genes of lignan biosynthesis. Beside the overexpression of the encoded proteins to study their functionality, downregulation of the expression of candidate genes is an approach to test their involvement in lignan biosynthesis. For this, we established an efficient and convenient transformation system with *Agrobacterium rhizogenes* for *Linum* species. *Linum* species are a rich source of different lignan structures which are accumulated in hairy roots as well. The expression of intron-hairpin-RNAi constructs in the hairy roots leads to the degradation of the mRNA of the target gene. We transformed shoot cultures with such a construct to downregulate the expression of pinoresinol-lariciresinol reductase (PLR), one of the first genes cloned for lignan biosynthesis. This gave the first genetic proofs for the involvement of a PLR in the biosynthesis of lignans with different general structures like podophyllotoxin, justicidin B and hinokinin.

S7-5

BIOSYNTHESIS OF LIPID RESORCINOLS AND BENZOQUINONES IN ISOLATED SECRETORY ROOT HAIRS OF *S. BICOLOR*

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While the primary functions of root hairs are to increase the root surface area and to aid plants in water and nutrient uptake, the root hairs of some plants have evolved the capacity to exude secondary metabolites. Sorghum (*Sorghum bicolor*) releases a substantial amount of phenolic lipids via their root hairs. The activity of the key enzymes involved in the biosynthesis of lipid resorcinols and benzoquinones was measured directly in isolated root hairs. The purified root hairs' preparation readily converted long chain acyl-CoA starter units to their corresponding lipid resorcinols. Optimum activity was with decanoyl-CoA, which yielded a 5-*n*-nonyl-resorcinol. The isolated root hair preparation also had high *S*-adenosyl-L-methionine-dependent *O*-methyltransferase activity, which catalyzes the methylation of several 5-alkyl-resorcinols. Optimum activity was with 5-*n*-pentyl-resorcinol. Isolated root hairs also exhibited hydroxylase activity (putatively a P450 monooxygenase) that reacted with the lipophilic 5-pentadecyl-resorcinol substrate. The *in situ* hydroxylase activity was low relative to the other enzymes studied, but was still detectable in isolated root hairs. Thus, sorghum root hairs possess the entire metabolic machinery necessary for the biosynthesis of lipid resorcinols. It also demonstrates that root hairs can function as specialized cells for the production of bioactive secondary metabolites.

S8-1

CONTROL OF METABOLISM IN A SINGLE CELL TYPE

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Plants produce an enormous diversity of specialized compounds, such as terpenoids and phenylpropanoids, which play important roles in plant defense and pollen and seed dispersal. The number and types of specialized compounds produced by any plant is unique, producing considerable variation even within the same species and consequently resulting in distinct chemotypes. The aromatic herb sweet basil (Ocimum basilicum L.) possesses chemotypes with distinctive chemical profiles, differentiated by the aromatic and medicinal compounds produced in peltate glandular trichomes. We have used a biochemical genomics-based approach to investigate the formation of specific compounds and the control of metabolism in basil, and to identify the corresponding genes involved. We have compared metabolite profiling data with digital gene expression analysis of chemotype-specific EST databases, which was supported by quantitative real time PCR, and with proteomics data. These investigations revealed that a chemotype's transcriptional and proteomic profiles (i.e., the necessary enzymatic components of the desired pathway) often correspond to what one would expect for its respective metabolic profile, leading to identification of candidate genes for specific enzymatic transformations or regulatory roles. Moreover, the transcription of other, non-enzymatic, proteins such as transcription factors appears to be differentially regulated between chemotypes, indicating a possible mechanism to explain the metabolic differences observed between chemotypes. Proteomics investigations, especially phosphopeptide analysis, suggested that post-translational modification of specific proteins, including many enzymes in the MEP/terpenoid and shikimate/phenylpropanoid pathways, may also play a role in regulating metabolism in these plants.

S8-2

LASER MICRODISSECTION AND PRESSURE CATAPULTING (LMPC): ENABLING TECHNOLOGIES FOR PLANT RESEARCH

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The increasing availability of genomic information and resources has prompted researches to investigate molecular processes in plants on a consistently decreasing scale. Gene, protein as well as metabolite analysis is now aimed to reveal further details at tissue- and even cell-specific levels. Therefore high-precision tools are needed to allow dissection of cellular, and even subcellular structures without compromising the integrity of biomolecules for downstream analysis.

The Zeiss PALM® (Positioning & Ablation with Laser Microbeams) is a modular microdissection and cell manipulation system. Utilizing a pulsed non-heating UV-A laser, the system allows precise and safe laser dissection of large tissue areas (> 1mm) to small sub-cellular structures (< 1µm). Following microdissection, Laser Pressure Catapulting safely ejects dissected cells into a collecting device, hence providing a non-contact tool for contamination free sample collection.

Using this approach in combination with 454 DNA sequencing researches have isolated cDNA from shoot apical meristems and generated >261,000 ESTs, >70% more than previously from hand-dissected material. Sequencing revealed >25,000 gene sequences, many of which lacked homologues in other species (Emrich et al. 2007). Other researched used LMPC successfully for cell specific quantitative gene expression analysis in giant root cells in response to parasite invasion (Ramsay et al. 2004). Importantly, LMPC has recently been used for the first time to analyze protein and metabolite content from as little as 100 *Arabidopsis* vascular bundles (Schad et al. 2005). These studies show that PALM laser microdissection is an enabling technology that facilitates cell specific molecular analysis from a large variety of plant tissues.

S8-3

DEVELOPMENT AND EXPERIMENTAL TESTING OF KINETIC MATHEMATICAL MODELS FOR ISOPRENOID ESSENTIAL OIL BIOSYNTHESIS IN MINT

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The monoterpenoid essential oils of peppermint (*Mentha* x *piperita*) are synthesized and stored in specialized glandular trichomes (oil glands) on aerial surfaces. Based upon data regarding the patterns of relevant transcripts, enzyme activities and metabolites, and an assessment of the kinetic properties of the biosynthetic enzymes, we developed a mathematical model of monoterpene biosynthesis in peppermint oil glands. Using an approach that includes mathematical simulations and experimental testing in an iterative cycle, we can formulate and test non-trivial hypotheses regarding the factors controlling essential oil yield and composition. For example, we were interested in understanding the cause for the accumulation of the monoterpene pathway intermediate pulegone PUL and the pathway byproduct menthofuran (MF) under certain environmental conditions (low ambient light, low water, high night temperatures). Our simulations indicated that the observed monoterpene profiles could be explained if an inhibitory effect of MF on pulegone reductase (PulR), the enzyme that converts the intermediate PUL into the major essential oil component menthone, was assumed. To test the validity of our model predictions, we expressed recombinant PulR in *E. coli*, purified the recombinant enzyme and tested the effects of MF on *Km* and *Vmax* properties. Our kinetic analyses established that MF acts as a competitive inhibitor, thus competing with PUL for the substrate binding site in PulR. Since the *Ki* properties determined for the inhibition of PulR by MF was relatively high, we evaluated the intracellular concentration of MF in the essential oil producing cells of peppermint. We isolated oil gland secretory cells from peppermint leaves, removed the external oil, and subjected them to steam distillations. MF turned out to accumulate to millimolar levels in secretory cells obtained from peppermint plants grown under stress conditions but not in controls maintained under regular growth conditions. These results illustrate the utility of iterative cycles of mathematical modeling and experimental testing.

S8-4

SYNTHESIS AND FUNCTION OF VOLATILE TERPENES: NEWS FROM THE *ARABIDOPSIS* MODEL

Dorothea Tholl (Virginia Polytechnic Institute and State University)

Volatile terpenes have received much attention in recent years as mediators of plant-organism/environment interactions. Research on model plant systems has led to significant progress in integrating metabolism, regulation and ecological roles of terpene volatiles. However, challenges remain in elucidating the pathway for the biosynthesis of those terpene volatiles with important biological function such as the insect-induced homoterpenes. Furthermore, our knowledge of activities of volatile terpenes at the cellular level prior to emission is rather limited. I will present recent advances in both topics based on our studies with the *Arabidopsis* model:

(1) We have gained insight in the biosynthesis and regulation of the C_{16}-homoterpene TMTT (4,8,12-trimethyl-1,3,7,11-tridecatetraene), which represents one of the most commonly insect-induced volatiles with crucial function in indirect plant defense.

(2) Using a transgenic approach, we have obtained new evidence that sesquiterpenes – similar to isoprene – enhance plant resistance against oxidative stress. Our results suggest a general role of volatile terpenes as endogenous regulators of reactive oxygen species under abiotic and biotic stress conditions.

S8-5

IDENTIFICATION AND CHARACTERIZATION OF PUTATIVE FLAVONOID GLUCOSYLTRANSFERASES FROM C*ITRUS PARADISI*

Daniel K. Owens and Cecilia A. McIntosh (Department of Biological Sciences, East Tennessee State University, Johnson City, TN, 37614)

Flavonoids are a diverse group of ubiquitous natural products in higher plants. Flavonoids are associated with essential physiological roles *in planta* and have activities that are agriculturally and pharmacologically important. The addition of sugars is a predominant modification reaction in flavonoid biosynthesis affecting the solubility, stability, and subsequent availability of metabolic products. Flavonoid glycosylation is of particular interest in grapefruit where up to 70% of the dry weight of very young fruit consists of flavanone glycosides. Flavonoid glycosides convey taste characteristics in citrus making flavonoid glucosyltransferases interesting targets for biotechnology applications in these species. To investigate glycosylation of flavonoids in *Citrus paradisi*, putative glucosyltransferase clones are being isolated from young leaf cDNA employing RACE with degenerate primers designed against the plant secondary product glucosyltransferase (PSPG) box signature motif. Complete gene sequences are identified by primer walking, recombinantly expressed in a bacterial system, and purified utilizing a 6X His tag. Identified recombinant enzymes are tested for substrate usage and thoroughly enzymatically characterized. While new putative glucosyltransferases continue to be sought, appropriate conditions for the production of soluble Cp_PGT2, Cp_PGT3, and Cp_PGT7 in *E. coli* have been established. Subsequently, glucosyl transferase activity for Cp-PGT3 and Cp_PGT7 has been verified with different flavonoid substrates. Identification of substrate glycosylation position and enzymatic characterization is ongoing.

S8-6

TRICHLOROETHYLENE INDUCES FORMATION OF STILBENOID COMPOUNDS AND ANTIOXIDANT ACTIVITY IN PEANUT ROOTS

Fabricio Medina-Bolivar, Cesar Nopo-Olazabal, Sivakumar Ganapathy, Luis Nopo-Olazabal, Robyn Hannigan, Kelly Redeker, Argelia Lorence, Christopher Purnell, Rodney S. Harris, Scott Simeon (Arkansas Biosciences Institute, Arkansas State University)

Trichloroethylene (TCE) is a widespread environmental pollutant commonly found in superfund sites. In humans, TCE toxicity is mediated by the induction of oxidative stress. However, in plants, the mechanisms of TCE toxicity and/or detoxification have not been fully elucidated. To study if antioxidant mechanisms are involved in the plant's response to TCE, we used hairy roots as our experimental system. We have shown that peanut hairy roots can be induced to produce antioxidant stilbenes (i.e. resveratrol and derivatives) after exposure to sodium acetate (Medina-Bolivar et al., Phytochemistry, *in press*). In this study, these cultures were exposed to 2000 ppm TCE for 24 hours and TCE, stilbenes and antioxidant activity were measured in the medium at 0, 6, 12, and 24 hours after TCE treatment. GC-MS analysis showed that TCE was rapidly taken up by the roots. In addition, the antioxidant activity increased 2-fold within 24 hours after exposure to TCE. For stilbene studies, ethyl acetate extracts of the medium were analyzed by HPTLC, HPLC with coupled PDA/fluorescence detection and HPLC-ESI-MS. These studies showed that TCE induced one major stilbenoid molecule at *m/z* 241. Interestingly, the prenylated resveratrol derivatives, usually found upon sodium acetate elicitation, were not induced by TCE. Our results indicate that stilbenoid production is a major mechanism of defense against environmental pollutants in peanut roots and that different types of stilbenes are induced depending upon the type of stress.

S8-7

HPLC-MS SCREENING FOR RESVERATROL AND ITS DERIVATIVES IN HAIRY ROOT CULTURES OF PEANUT

Ganapathy Sivakumar, Jose Condori, Robyn Hannigan, Maureen Dolan, and Fabricio Medina-Bolivar (Arkansas Biosciences Institute, Arkansas State University)

Resveratrol and its prenylated derivatives including arachidin-1, arachidin-3 and isopentadienylresveratrol belong to the stilbene class of biophenolic molecules and are known to have potent anti-inflammatory, antioxidant and anti-cancer properties. In light of the increased attention in naturally occurring bioactive stilbenes as alternative chemopreventive and antioxidant agents in human health management, there is interest in developing a systematic, culture-based, bioproduction system for these valuable phytomolecules. We have previously shown that hairy root cultures of peanut (*Arachis hypogaea*) can be established and elicited with sodium acetate to produce and secrete resveratrol and its related derivatives (Medina-Bolivar et al. 2007, Phytochemistry*, in press*). In this study HPLC-MS was used in establishing a quantitative and qualitative profile of resveratrol and its derivates secreted from elicited hairy root cultures. Medium extracted with ethyl acetate and dried was then dissolved in methanol and analyzed by HPLC-ESI-MS. In negative ionization mode, three major molecules were detected and identified as *trans*-resveratrol (*m/z* 227), arachidin-1 (*m/z* 311) and arachidin-3 (*m/z* 295). In addition, the resveratrol derivatives piceatannol (*m/z* 243) and isopentadienylresveratrol (*m/z* 293) were found but at lower quantities. These compounds were also confirmed by both HPTLC and HPLC coupled with PDA and fluorescence detectors. Our results highlight the potential of the hairy root technology for consistent and controlled production of extracts enriched in these valued, bioactive stilbenes with numerous health and wellness applications.

PME-1 (Graduate Student)

CHEMICAL GENOMICS TO DECIPHER GENES INVOLVED IN ROOT SECRETIONS

Meredith L. Biedrzycki and Harsh P. Bais (Department of Plant and Soil Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711)

Due to their involvement in an abundance of biological interactions, root secretions are beginning to gain attention in the field of rhizosphere biology. Although previous research has looked at the components of root secretions and situations where root secretions are involved, the genetic basis of how root secretions are regulated has not been considered. To this end, we envisaged to exploit a high-throughput chemical genomics assay using a model plant system *Arabidopsis thaliana* to determine the genes responsible for root secretions. Additional confocal microscopy techniques were used to decipher the mechanism by which root secretions are transported through and out of root cells. Chemical genomics (large-scale chemical genetics) aims for developing high-throughput assays, through use of diverse commercial small molecule chemical libraries, for detection of phenotypes of interest. Genome-sequenced organisms, such as *A. thaliana*, and micro-arrays are essential to chemical genomics for identification of the target genes and their functions. Recently, chemical genomics is becoming recognized as a unique and essential tool for plant biologists studying various metabolic pathways. To facilitate this chemical genomics screen, specifically phenol and flavonoid secretions of *A. thaliana* were targeted. To date, six chemicals from the small molecule library have been identified as increasing both phenol and flavonoid root secretions in *A. thaliana* seedlings. Future work will include verification of these “hit” chemicals through a second screen utilizing other analytical techniques. Root secretion gene(s) candidates will be targeted through the use of micro-array analysis of plants treated with “hit” chemicals. Based on the microarray data, T-DNA knockout mutants will be examined to verify gene function.

PME-2 (Graduate Student)

CLONING AND CHARACTERIZATION OF A NOVEL STILBENE SYNTHASE FROM PEANUT

Jose Condori and Fabricio Medina-Bolivar (Arkansas Biosciences Institute, Arkansas State University)

Stilbenes are specialized metabolites derived from the phenylpropanoid-acetate pathway that have been described in taxonomically unrelated species including grape, peanut and pine. The most well-known and well-characterized stilbene is resveratrol, whose biosynthesis has, as the final step, the condensation of one molecule of *p*-coumaryl-CoA and three molecules of malonyl-CoAs catalyzed by resveratrol synthase. Chalcone synthase, an ubiquitous enzyme, also uses the same substrates as resveratrol synthase. Previous studies have shown the existence of up to three resveratrol synthase genes in peanut (*Arachis hypogaea*). However, only partial DNA sequences are available for two of them. Recently, we have shown that hairy root cultures of peanut (*Arachis hypogaea*) cv. Andru II can be induced to produce resveratrol and derivatives upon elicitation (Medina-Bolivar *et al.*, Phytochemistry, 2007, *in press*). In the present study, the entire cDNAs for three putative stilbene synthases and one chalcone synthase gene were cloned from peanut hairy roots using primers based on peanut DNA and cDNA sequences available at NCBI. The primer sequences were designed in conserved regions to distinguish between resveratrol synthase and chalcone synthase. Our results show the presence of a putative stilbene synthase gene, which is 92.5% identical (at amino acid level) to chalcone synthase. Characterization of this novel stilbene synthase gene in a transient eukaryotic expression system is currently underway. These studies will advance our understanding of the diversity of stilbene synthases and may contribute to new metabolic engineering strategies for the production of stilbenoid compounds.

PME-3 (Graduate Student)

REGULATION OF MONOTERPENOID ESSENTIAL OIL BIOSYNTHESIS IN PEPPERMINT

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The integration of both mathematical modeling and experimental testing is emerging as a powerful tool for improving our understanding of plant metabolism. In this study, we present the development of a kinetic model for monoterpenoid essential oil biosynthesis in peppermint (*Mentha* x *piperita*). In particular, we were interested in assessing the cause for the accumulation of the monoterpene pathway intermediate pulegone (Pul) and the pathway byproduct menthofuran (MF) under certain environmental conditions (low ambient light, low water, high night temperatures). Our simulations indicated that the observed monoterpene profiles could be explained if an inhibitory effect of MF on pulegone reductase (PulR), the enzyme that converts the intermediate Pul into the major essential oil component menthone (M-one), was assumed. To test the validity of our model predictions, we expressed recombinant PulR in *E. coli*, purified the recombinant enzyme and tested the effects of MF on K_m and V_{max}. Our kinetic analyses clearly established that MF acts as a competitive inhibitor (K_i = 300 µM), thus competing with Pul for the substrate binding site in PulR. Since the K_i we determined for the inhibition of PulR by MF was relatively high, we evaluated the intracellular concentration of MF in the essential oil producing cells of peppermint. Thus, we isolated oil gland secretory cells from peppermint leaves, removed the external oil, and subjected them to steam distillations. MF turned out to accumulate to high levels (20 mM) in secretory cells obtained from peppermint plants grown under stress conditions but not in controls maintained under regular growth conditions. These results illustrate the utility of iterative cycles of mathematical modeling and experimental testing to elucidate the factors controlling flux through metabolic pathways.

PME-4

EFFECTS OF FUNGAL ELICITORS ON ISOFLAVONOID ACCUMULATION IN *PUERARIA LOBATA* CELL SUSPENSION CULTURE

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Kudzu (*Pueraria lobata)* produces important isoflavone compounds, which have many health and nutritional applications. Various fungal elicitors derived from six fungi were tested to stimulate culture growth and increase isoflavone production in kudzu cell suspension cultures. The fungal elicitor from *Rhizoctonia cerealis* significantly stimulated cell growth. However, many fungal elicitors significantly inhibited cell growth. The elicitor derived from *Fusarium oxysporum f.sp. Versinfectum* showed different effects on cell growth: a significant stimulating effect on cell growth in lower concentrations; and a significant inhibiting effect in higher concentrations. Some elicitors had no significant effects on cell growth, such as *Venturia pirina* and *F. moniliforme*. For isoflavone biosynthesis, some fungal elicitors inhibited isoflavonoid accumulation in *P. lobata* cell culture, such as elicitors from *F. oxysporum* and *F. moniliforme*. The fungal elicitors from *Pestalotiopsis* and *V. pirina* significantly stimulated isoflavonoid accumulation. Some elicitors have different effects on isoflavone accumulation, such as *F. oxysporum f.sp. Versinfectum* and *R. cerealis*, which showed significant stimulating or inhibiting effects at different concentrations. These results are helpful to select fungal elicitors for the improvement of isoflavone yield and further understanding of the interactions between fungal elicitors and plant cells.

PHP-1 (Graduate Student)

ISOLATION AND IDENTIFICATION OF ANTIADHESIVE URINARY METABOLITES FROM CRANBERRY JUICE

Christina M. Coleman[1], Daneel Ferreira[1], Amy B. Howell[2], Jess D. Reed[3], Christian G. Krueger[3] and Jannie P. J. Marais[4] ([1]University of Mississippi, [2]Rutgers University, [3]University of Wisconsin, and [4]National Center for Natural Products Research)

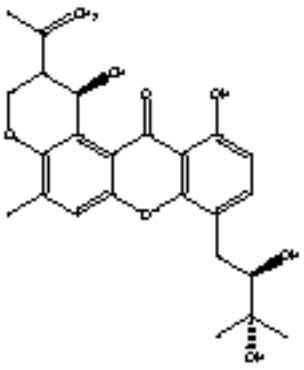
Compounds found in cranberry juice (*Vaccinium macrocarpon*) have been shown to prevent the adherence of P-fimbriated uropathogenic bacteria to uroepithelial cells *in vitro.* These compounds are therefore believed to be able to prevent urinary tract infections by preventing the adherence of such bacteria to the cells lining the urinary tract. Previous work by our collaborators has identified the active compounds from cranberry juice as proanthocyanidin trimers and oligomers which contain at least one A-type linkage. While administration of these compounds results in anti-adherence activity of urine following ingestion by both humans and swine, the active urinary metabolites from cranberry juice are currently unidentified. An adult female sow was therefore fed approximately 5 g lyophilized cranberry juice powder per kg body weight per day for three days prior to collection of urine via catheter. Feeding and collection was then continued for a week. A human red blood cell agglutination bioassay with uropathogenic P-fimbriated *E. coli* was used to identify bioactive urine fractions. Active compounds were then isolated using Sephadex LH-20, C-18, and other chromatography techniques. Identification and structural characterization were performed using NMR, MS and other spectroscopic techniques. Preliminary evidence supports the identification of the compounds as derivatives of A-type proanthocyanidin trimers or oligomers. Information on the isolation and structural characterization of the bioactive metabolites will be presented.

PHP-2 (Graduate Student)

PHYTOTOXIC COMPOUNDS FROM A NEW *EMERICELLA* SPECIES

Mario Figueroa[1], María del Carmen González[2] and Rachel Mata[1] ([1]Facultad de Química, and [2]Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City 04510, México)

The new fungus *Emericella* A-2 was isolated from a coral species collected in Cozumel, Mexico. Bioassay-directed fractionation of a phythotoxic dichoromethane extract (broth) led to the isolation of several xanthones. Their structures were elucidated by spectral and spectrometric analyses. Metabolite **1** caused significant inhibition of radicle growth of seedlings of *Amaranthus hypochondriacus* with IC_{50} 0.35 μM.



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Supported by CONACyT (CO1-018) and DGAPA (IN 208907).

PHP-3 (Graduate Student)

IMMUNOMODULATING PROPERTIES OF BIOACTIVE SECONDARY METABOLITES FROM CULTURE BROTH OF TWO MEXICAN STRAINS OF BASIDIOMYCETES

Patricia González Barranco[1], Lourdes Garza Ocañas[1], Oscar Torres Alanís[1], Rubén Luján Rangel[1], Mario César Salinas Carmona[1], Fortunato Garza Ocañas[2], and Xóchitl S. Ramírez Gómez[3] ([1]Facultad de Medicina, Universidad Autónoma de Nuevo León, [2]Facultad de Ciencias Forestales, UANL, [3]Facultad de Medicina, Universidad de Guanajuato)

Asian Basidiomycetes have biologically active (antitumoral) molecules which produce their effect by activating different immune responses in the host. In spite of the great number of mushroom species growing in Mexican forests, most of them have scarcely been investigated. In the present study, we evaluated the immunomodulating properties as well as the cytotoxicity of two mexican Basidiomycetes strains: *Suillus lakei* and *Lentinus lepideus*. These strains were cultivated *in vitro* and culture broth samples were taken at different growing times. The immunomodulating activity was evaluated according to Cunningham's technique in BALB/c mice, while the cytotoxicity was evaluated by the MTT test in Chang liver cells. Bioassay guided fractionation of the two-month culture broth samples was carried out and fractions were evaluated using the same parameters. *Suillus lakeii* induced a significant increase (124%) in the plaque-forming cell (IgM) production (immunomodulating effect). None of the strains was cytotoxic. The phytochemical analysis of the bioactive strain showed the presence of phenols.

PHP-4 (Graduate Student)

COMPARATIVE ANALYSIS OF THE PHYTOCHEMICAL CONSTITUENTS OF FRESH AND DRY LEAVES OF *GONGRONEMA LATIFOLIA* AND *VERNONIA AMYGDALINA*

Onyekachi O. Iroanya and Joy Okpuzor (Department of Cell Biology and Genetics, University of Lagos, Akoka, Yaba, Lagos, Nigeria)

Gongronema latifolia and *Vernonia amygdalina* are leafy vegetables abundant in the tropical forests of southwestern Nigeria and some parts of West Africa; they are used as spices for sauces and as medicinal plants. There are reports of their use in the treatment of diabetes, dysentery and antiparasitic properties. *Vernonia amygdalina* has been reported to have hypoglycaemic and antimalarial properties. The roots and stems are used as chewing sticks and the bitter taste has antimicrobial activity against oral microflora The use of medicinal plants in traditional healing is holistic; therefore, it is important that systematic studies be undertaken to identify the bioactive components of *Gongronema latifolia* and *Vernonia amygdalina* responsible for their healing properties.

Methanol extracts of fresh and dried samples of both plants were fractionated in butanol, chloroform, hexane, ethyl acetate and water to identify the active constituents. We report the following compounds being present in the crude methanol and fractionated extracts of *Gongronema latifolia* and *Vernonia amygdalina* - alkaloids, flavonoids, cardiac glycosides, free and bound anthraquinones, tannins and saponins. No cyanide was detected. The results of TLC and HPLC are also presented.

PHP-5 (Graduate Student)

FLUORIMETRIC ASSAY OF HISTONE DEACETYLASE INHIBITORS OF PLANT ORIGIN

Stanimira Krasteva, Elke Heiss, Verena Dirsch, Liselotte Krenn (University of Vienna, Centre of Pharmacy, Austria)

Histone deacetylase inhibitors (HDACi) have received high interest as anticancer agents only recently. By epigenetic chromatin remodeling, HDACi may negatively influence tumor growth e.g. by inhibition of angiogenesis or by increasing tumor cell immunogenicity [1]. In many clinical trials, effects of several HDACi on different tumors are studied at the moment [2]. The plant kingdom provides an almost infinite structural biodiversity. Thus, the aim of our study is to i) establish a fast method for the determination of HDAC activity, ii) compare HDAC inhibitory activity in different cell types and iii) identify potent and natural product-based inhibitors of HDAC activity. A fluorimetric enzyme assay was established to investigate HDAC activity and its modulation by different natural compounds. Nuclear extracts from human umbilical vein endothelial cells (HUVECs), rat vascular smooth muscle cells (VSMCs) and HeLa cells served as source of HDAC protein. Natural test compounds were selected according to their structural relationship to known HDAC inhibitors. Initial results showed that compounds such as several isoflavones, anthraderivates and capsaicines have a potential to inhibit histone deacetylase activity, although only at micromolar concentration. IC_{50} values were determined for aloin (150 µM), for sennosides A and B (180 µM and 220 µM) and capsaicine (400 µM). Further studies are warranted to find potent histone deacetylase inhibitors at nanomolar concentration.

Acknowledgements: We are grateful to Dr. Dan Sorescu, Emory University, USA, for helpful discussions.

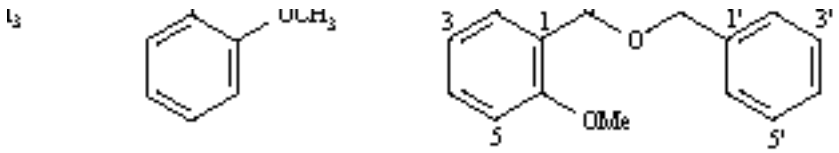
References: 1 Ogbomo, H. et al. (2007) FEBS Letters 581, 1317-1322; 2 Garber, K. Arbor, A. (2007) Nature Biotechnology 25, 17-18.

PHP-6 (Graduate Student)

ANTINOCICEPTIVE EFFECT OF SALICYLIC ACID DERIVATIVES FROM *BRICKELLIA VERONICIFOLIA*

Juan Francisco Palacios-Espinosa[1], Myrna Déciga-Campos[2] and Rachel Mata[1] ([1]Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City 04510, México. [2]Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México)

Oral administration (150-600 mg/kg) of a MeOH-CH_2Cl_2 (1:1) extract of *Brickellia veronicifolia* (Kunth) Gray (Asteraceae) produced a significant antinociceptive effect when tested in mice using the writhing and the hot-plate models. From the active extract, several benzoic acid and benzyl benzoates derivatives were isolated, including 2-hydroxy-6-methoxybenzoic acid (**1**), 2-methoxybenzoic acid (**2**) and benzyl 2,6-dimethoxybenzoate (**3**). Compounds **1** and **2** (1-100 mg/kg, p.o.) significantly increased the hot-plate latency in comparison to vehicle-treated mice. Morphine (2.5-5 mg/kg) was used as positive controls. Compound **3** was less active than the benzoic acid derivatives.



(**1**) (**2**) (**3**)

Supported by CONACyT (CO1-018) and DGAPA (IN 208907).

PHP-7 (Graduate Student)

ANTIHYPERGLYCEMIC EFFECTS OF *BRICKELLIA VERONICIFOLIA* IN STREPTOZOCIN-INDUCED DIABETIC RATS

Juan Francisco Palacios-Espinosa, José Antonio Guerrero-Analco and Rachel Mata (Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City 04510, México)

Brickellia veronicifolia (Kunth) Gray (Asteraceae) is one of the species most widely commercialized in Mexico to cure many gastrointestinal disorders, as well as arthritis pain and diabetes. Oral administration of a CH_2Cl_2-MeOH (1:1) extract (10, 100 and 300 mg/kg of body weight) and essential oil (10 mg/kg of body weight) prepared from the aerial parts of the plant significantly reduced blood glucose levels in normoglycemic and streptozotocin (STZ)-induced diabetic rats. Furthermore, in an OGTT (Oral Glucose Tolerance Test) the extract at a dose of 100 mg/kg induced a decrease in blood glucose levels in normal rats. These findings support the traditional use of the plant to treat diabetes and suggest that its antihyperglycemic effect might be due to an interference with glucose absorption.

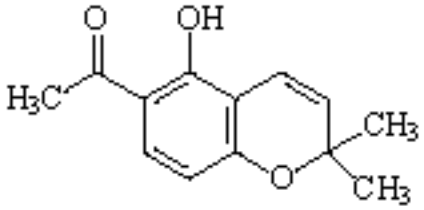
Supported by CONACyT (CO1-018) and DGAPA (IN 208907).

PHP-8 (Graduate Student)

PHYTOTOXIC POTENTIAL OF *BRICKELLIA CAVANILLESII*

Juan Francisco Palacios-Espinosa, Martha Adriana Leyte-Lugo and Rachel Mata (Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City 04510, México)

A CH_2Cl_2-MeOH (1:1) extract prepared from the aerial parts of *Brickellia cavanillesii* (Asteraceae) inhibited radicle growth of *Amarathus hypochondriacus* (IC_{50}=276 µg/mL) when tested by the petri dish assay. Accordingly, the phytotoxic extract was selected for bioassay-guided fractionation. This process led to isolation of the chromene 1. Compound 1 inhibited radicle elongation of *A. hypochondriacus* seedlings. The calculated IC_{50} value of 1 was 0.275 µM.



(**1**)

Supported by DGAPA (IN 212005).

PHP-9 (Graduate Student)

PHYTOTOXIC ACTIVITY AND CONFORMATIONAL ANALYSIS OF THYMOL ANALOGS FROM *HOFMEISTERIA SCHAFFNERI*

Araceli Pérez-Vásquez[1], Edelmira Linares[2], Robert Bye[2], Carlos Cerda-García-Rojas[3] and Rachel Mata[1] ([1]Facultad de Química and [2]Instituto de Biología, Universidad Nacional Autónoma de México, México City, 04510, México, [3]CINVESTAV-IPN, México DF, México)

Hofmeisteria schaffneri (A. Gray) R. M. King & H. Robinson (Asteraceae) is an endemic Mexican medicinal herb. Continuing with the investigation of the phytotoxic potential of *H. schaffneri*, bioassay-guided fractionation of the phytotoxic extracts of a new collection of this plant led to isolation of one new thymol derivative and seven known compounds. The new compounds were characterized by spectroscopic methods. In addition, the conformational behavior of the isolates was determined using density functional theory calculations at B3LYP/DGDZVP level. The phytotoxic activity of the extracts and isolated compounds was assessed on *Amaranthus hypochondriacus*.

Supported by CONACyT

PHP-10 (Graduate Student)

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *HOFMEISTERIA SCHAFFNERI* (A. GRAY) R. M. KING & H. ROBINSON (ASTERACEAE)

Araceli Pérez-Vásquez[1], Edelmira Linares[2], Robert Bye[2], Santiago Capella[1] and Rachel Mata[1] ([1]Facultad de Química and [2]Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, 04510, México)

Capillary GC/MS analysis has been applied to the analysis of the essential oils obtained from *Hofmeisteria schaffneri* (Asteraceae) harvested at five different periods during a year. Thirty four compounds were identified representing circa 90 % of the total amount. The oils showed synchronized patterns of variation during the different seasons with thymyl angelate (1) and thymyl isovalerate (2) always being the main constituents. The amount of compounds 1 and 2 ranged from 34 to 40 % and 10 to 18 %, respectively. The phytotoxic effect of the five oils, determined by measuring radicle growth inhibition of seedlings of *Amaranthus hypochondriacus*, remained constant with IC50 ranging from 120 to 123 µg/mL.

Supported by CONACyT (Project 45814-Q)

PHP-11 (Graduate Student)

RHODODENDRON SP. EXTRACTS INFLUENCE EUKARYOTIC CELL DIVISION

Ruxandra Popescu[1,2], Sibylle Madlener[2], Nicole Stark[2], Georg Krupitza[2] and Brigitte Kopp[1] ([1]University of Vienna, Centre of Pharmacy, Austria [2]Medical University of Vienna, Austria)

Members of the *Ericaceae* family, such as *Rhododendron* sp. and *Kalmia* sp., are known as toxic plants. This study aims to asses the cytotoxicity of the European species *Rhododendron kotschyi*, *R. ferrugineum* and *R. hirsutum* on eukaryotic cells. Dried leaves were successively extracted in petroleum ether, dichloromethane, ethyl acetate, methanol and water. *R. kotschyi* and *R. ferrugineum* extracts were first screened by phytobiological cell division assessment. For this, *Triticum* sp. rootlets were exposed to extracts in different concentrations and the evolution of rootlet length in time was observed. Microscopy of the rootlet after 24 hours of contact with the extract was also done. The ethyl acetate and methanol extracts showed a strong inhibition of root growth with frequent metaphases and also abnormal anaphases and telophases detected by microscopy. Further on, ethyl acetate extracts of *R. kotschyi*, *R. ferrugineum* and *R. hirsutum* were tested on human leukaemia HL-60 cells in concentrations ranging from 0.5 to 20 mg/ml. A proliferation assay was coupled with an apoptosis assay which uses double staining with fluorescent dyes. The first dye, Hoechst, binds chromatin, emits blue fluorescence and crosses intact cell membrane, being a marker for apoptosis. The second dye, propidium iodide, also binds chromatin, emits red fluorescence and cannot cross the intact cell membrane, pointing to necrosis. After 72 hours, the cell proliferation decreased with an increase in extract concentration for all three species, while cell apoptosis and necrosis increased. *R. hirsutum* ethyl acetate extract proved to be the most active, followed by *R. kotschyi* and *R. ferrugineum*. In conclusion, *R. kotschyi*, *R. ferrugineum* and *R. hirsutum* leaf extracts show activity on eukaryotic cell division suggesting a possible source of antineoplastic compounds.

PHP-12 (Graduate Student)

TOXICITY AND ESSENTIAL OIL COMPOSITION OF *POLIOMINTHA LONGIFLORA*

Isabel Rivero Cruz[1], Robert Bye[2] and Rachel Mata[1] ([1]Departamento de Farmacia, Facultad de Química, and [2]Instituto de Biología, Universidad Nacional Autónoma de México, México, DF 04510, México)

Poliomintha longiflora A. Gray (Lamiaceae) is extensively commercialized as substitute of common (*Origanum vulgare* L.) or Mexican (*Lippia graveolens* HBK) oreganum. Furthermore, *P. longiflora* is broadly exported to the US for use as condiment. Acute toxicity analysis of the extract and essential oil of *P. longiflora* using the Lorke procedure revealed that the plant is extremely toxic to mice. The LD_{50} of the extract was less than 2.0 mg/kg. During the first phase of the evaluation, all animals administrated with 10, 100 or 1000 mg/kg of the extract of *P. longiflora* died; the treatments also provoked fibrous nodules in the back and neck of the animals which died two or three days after the administration of the crude extract. During the second phase of the experiment, the same trends of effects were observed. Therefore, it is important that National Health Authorities notify local consumers as well as national and international traders about the risk of using this plant, at least until further studies are carried out. GC-MS and HPLC analyses of the essential oil indicated that the major constituents are a series of thymol analogs.

Supported by CONACyT (CO1-018) and DGAPA (IN 208907).

PNP-1 (Undergraduate Student)

ISOLATION, ANALYSIS, AND EXPRESSION OF PGT2 AND PGT4, PUTATIVE FLAVONOID GLUCOSYLTRANSFERASE CLONES FROM *CITRUS PARADISI* LEAVES

Leslie B. Epling*, Shannon McConnell*, Josephat Asiago*, Daniel K. Owens, and Cecilia A. McIntosh (East Tennessee State University; *authors contributed equally).

Flavonoids are one of the major groups of plant natural products and many exist in glycosylated form. Well over 100 putative secondary product glucosyltransferase (GT) genes have been identified from various plants but only a small number have been characterized. The majority of flavonoid GTs characterized to date belongs to the 3-O-GT group (act on flavonols, anthocyanins). Grapefruit is known for high levels of accumulation of flavanone-7-O-glycosides as well as flavone-7-O-glycosides, flavonol-7-O and 3-O glycosides, etc. It has been chosen as our model plant system in order to increase diversity of information to contribute to structure/function analysis of GTs. PGT2 and PGT4 were obtained by SMART-Race RT-PCR using primers designed against a conserved region of the PSPG box and subsequent design of gene specific primers (GSPs) to obtain full-length sequences by primer walking. Full length coding regions were obtained by designing GSPs to the 5' and 3' ends of each clone and PCR products TOPO-TA cloned for amplification. For cloning into pCD1 expression vector, the 5' end of PGT2 was modified to incorporate an NcoI site (the only available 5' cloning site in pCD1) and the 3' end to contain an XhoI site; it has been cloned into pCD1 and protein expression is being optimized. PGT4 contained internal NcoI sites, therefore, the 5' end was modified to contain a BsaI site producing an NcoI compatible overhang and the 3' end was modified to contain an XhoI site. PGT4 is currently being subcloned into pCD1. Once protein expression is optimized, the PGTs will be tested for flavonoid GT activity.

PNP-2 (Undergraduate Student)

ROLES OF CONSERVED SERINE AND TYROSINE RESIDUES IN THE ACTIVE SITE OF TYROSINE AMMONIA-LYASE: CONTRIBUTIONS TO CATALYSIS, LIGAND BINDING, AND COFACTOR PROCESSING

Amy C. Schroeder, Sangaralingam Kumaran, Leslie M. Hicks, Rebecca E. Cahoon, Coralie Halls, Oliver Yu, and Joseph M. Jez. (Donald Danforth Plant Science Center, 975 N. Warson Rd., St. Louis, MO 63132)

Tyrosine ammonia-lyase (TAL) catalyzes the conversion of L-tyrosine to *p*-coumaric acid using a 3,5-dihydro-5-methylidene-4H-imidazole-4-one (MIO) prosthetic group. In bacteria, TAL is used for production of the photoactive yellow protein chromophore and for caffeic acid biosynthesis in certain actinomycetes. This enzyme is also valuable for metabolic engineering applications requiring *p*-coumaric acid. Here we biochemically examine wild-type and mutant forms of the TAL from *Rhodobacter sphaeroides* (RsTAL). Kinetic analysis of RsTAL shows that the enzyme displays a 90-fold preference for L-tyrosine versus L-phenylalanine as a substrate. The pH-dependence of TAL activity with L-tyrosine and L-phenylalanine reveals a common protonation state for catalysis, but indicates a difference in charge-state for binding of either amino acid. Site-directed mutagenesis demonstrates that Tyr60, Ser150, and Tyr300 are essential for catalysis. Mutation of Ser150 to an alanine abrogates formation of the MIO prosthetic group, as shown by mass spectrometry, and prevents catalysis. The Y60F and Y300F mutants were inactive with both amino acid substrates, but bound *p*-coumaric and cinnamic acids with wild-type affinity. Spectroscopic analysis of MIO•dithiothreitol adduct formation shows that the reactivity of the prosthetic group is not altered by mutations of Tyr60 or Tyr300. The mechanistic roles of Ser150, Tyr60, and Tyr300 are discussed in relation to the recently published three-dimensional structure of RsTAL.

PNP-3 (Undergraduate Student)

STRUCTURE/FUNCTION STUDIES AND PROTEIN ENGINEERING OF ATP-DEPENDENT PEPTIDE LIGASES

Kiani A.J. Arkus, Katherine Herrera, Rebecca E. Cahoon, and Joseph M. Jez (Donald Danforth Plant Science Center, St. Louis, MO)

Glutathione is found in mammals, plants, and bacteria and is synthesized by glutathione synthetase (GS). Some plants synthesize glutathione analogs in which β-alanine replaces glycine. The chemical diversity of these molecules suggests that the substrate specificity of the GS-like peptide ligases differs from GS, but the molecular basis for this variety remains unclear. To better understand the reaction mechanism of *Arabidopsis* GS (AtGS) site-directed mutagenesis was performed. Residues interacting with bound magnesium ions (E148, N150, E371, and E429) were essential for activity. K313 and K367 were important for ATP binding. Kinetic analysis suggests that R132 provides a key catalytic role. To define the determinants of substrate specificity, we compared AtGS (glycine specific) with *Glycine max* (soybean) homo-glutathione synthetase (GmhGS; β-alanine specific). Sequence alignments suggest that two active site residues (AtGS: A466 and A467; GmhGS: L466 and P467) modulate substrate preference between glycine and β-alanine. The interconversion of AtGS to GmhGS and vice versa is described. GmhGS displays a 700-fold preference for β-alanine, whereas, the double mutant accepts either substrate with equal efficiency. This suggests that differences outside the active site further define substrate specificity in this family of enzymes, and emphasizes the need for crystallographic studies.

PNP-4 (Undergraduate Student)

A SURVEY OF SPHINGOLIPIDS IN MEMBERS OF THE VIRIDIPLANTAE

Anna Locke, Jonathan E. Markham and Jan G. Jaworski (Donald Danforth Plant Science Center, St. Louis, Missouri, USA)

Sphingolipids are unique, amide-acyl lipid compounds that have assumed specialized roles in cellular signaling and membrane sorting. Sphingolipids consist of an amino-acylalcohol or long chain base (LCB) group amide linked to a fatty acid to produce the ceramide structure which may then be glycosylated to produce an array of more complex sphingolipids. Thought to be universal within the *Eukaryota*, sphingolipids of the higher plants have been shown to be quite different compared to sphingolipids of the *Metazoa* or fungi. For example, animals and yeast typically contain two LCBs in their sphingolipids whereas plants may have up to six different LCB variants. In order to gain an evolutionary perspective with regard to the role of sphingolipids in plants we have undertaken a survey of the sphingolipid content of representative members from different orders of the *Viridiplantae*. The LCB component of the sphingolipids in these species have been measured by hydrolysis of entire tissue samples, extraction of the LCBs, fluorescent derivitization and separation, identification and quantification by HPLC. The results and groupings from this analysis will be presented and select species identified for more detailed description of their sphingolipid content

PNP-5 (Undergraduate Student)

CORRELATION BETWEEN EXPRESSION OF ACYLTRANSFERASES AND DIVERSITY OF COMPOUNDS CAUSING PUNGENCY IN *CAPSICUM* VARIETIES

James Moten, Laura Hernandez, and Mary O'Connell (Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM, 88003 USA)

This project seeks to identify and determine the relative abundances of six capsaicinoids (capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin, and norhomodihydrocapsaicin) within fruits of varying pungency from the genus *Capsicum*. In particular, we are looking for amino acid diversity in candidate capsaicinoid synthase enzymes that correlate with the fatty acyl diversity in individual capsaicinoids. Twelve varieties of chiles were collected in late summer and dried, after which capsaicinoids were extracted from the tissue in acetonitrile. Analysis of the extracts was accomplished by means of gas chromatography coupled to an electron ionization mass spectrometer. Nucleic acid primers were designed to analyze the gene predicted to synthesize capsaicinoids. Clones of this gene from twelve varieties of chiles are under investigation in an attempt to correlate variation in capsaicinoid profiles with variation in the acyltransferase genes.

This work was supported in part by NIH Grants #GM07667-29 and RISE GM61222.

PNP-6 (Graduate Student)

ISOLATION AND IDENTIFICATION OF THE PUTATIVE GLUCOSYLTRANSFERASE PGT8 FROM *CITRUS PARADISI*

J.K. Cooke[1,2], D. Owens[1], and C. McIntosh[1,3] ([1]Department of Biological Sciences, [2]Department Health Sciences, and [3]School of Graduate Studies, East Tennessee State University)

The isolation of the putative glucosyltransferase clone PGT8 and its resolution from other glucosyltransferases from *Citrus paradisi* (grapefruit) are described. This clone was obtained by designing two primers based on the sequence of the limonoid glucosyltransferase found in *Citrus unshiu*. These primers were designed so that PCR products would encompass the signature Plant Secondary Product Glucosyltransferase (PSPG) domain. Forward primer JC2 was taken from an area of the sequence approximately 50 base pairs ahead of the PSPG box. Reverse primer JC4 was taken from an area of the sequence approximately 100 base pairs after the PSPG box toward the 3' end. A PCR reaction with cDNA from *C. paradisi* yielded a fragment approximately 500 base pairs in length. This fragment was cloned into TOPO vector and plasmids were isolated from the cells, and sent for sequencing. Analysis of sequences confirmed the presence of the PSPG box and showed >90% identity, at both the nucleotide level and the amino acid level, with the limonoid GT in *C. unshiu*. New primers CSP53F and CSP54R were designed based on the 3' and 5' ends of the limonoid gene in C. unshiu. These primers also contain modified ends specific to enzymes PciI and BamHI to facilitate cloning into pCD1. The new primers were used in a PCR reaction in order to obtain the entire sequence for this putative GT. The PCR products have been cloned into TOPO vector and plasmids have been isolated. Sequencing to confirm clone identity is in progress, after which the insert will be cloned into pCD1 expression vector for further analysis.

PNP-7 (Graduate Student)

ISOLATION AND CHARACTERIZATION OF TWO CYTOCHROME P450s FROM THE BIOSYNTHESIS OF STYLOPINE

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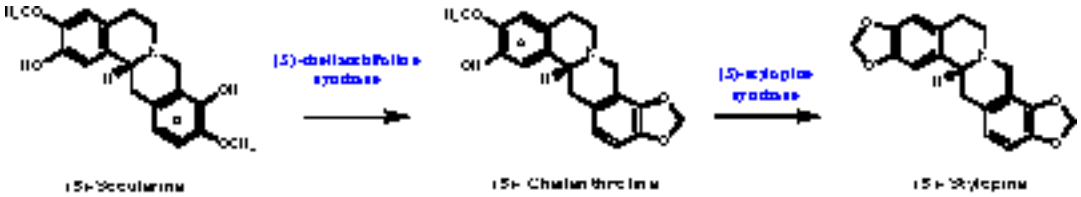
Argemone mexicana L. (Papaveraceae) commonly known as prickly poppy, is a traditional medicinal plant used for the treatment of fever, pain, diarrhea, cutaneous infections and cancer. Phytochemical studies identified different benzophenanthridine, protoberberine, and protopine type alkaloids, some of them with antimicrobial activity and anti-tumor properties. In an effort to identify and characterize genes involved in isoquinoline alkaloid biosynthesis, a cDNA library was constructed. A total of 1,600 expressed sequence tags (ESTs) were sequenced generating 1,255 unique sequences. Using this information, two methylenedioxy bridge-forming enzymes (CYP719) were isolated and heterologously expressed in insect cells. The first one, (*S*)-cheilanthifoline synthase (CYP719A14) recognizes (*S*)-scoulerine as substrate to form (*S*)-cheilanthifoline and the second one (*S*)-stylopine synthase (CYP719A13) catalyzes the biosynthesis of (*S*)-stylopine from (*S*)-cheilanthifoline. In both cases, high substrate specificity was observed. In this work, the enzymatic characterization and amino acid sequence comparison are reported.



PNP-8 (Graduate Student)

IDENTIFICATION OF CYP450 719B1 AS SALUTARIDINE SYNTHASE

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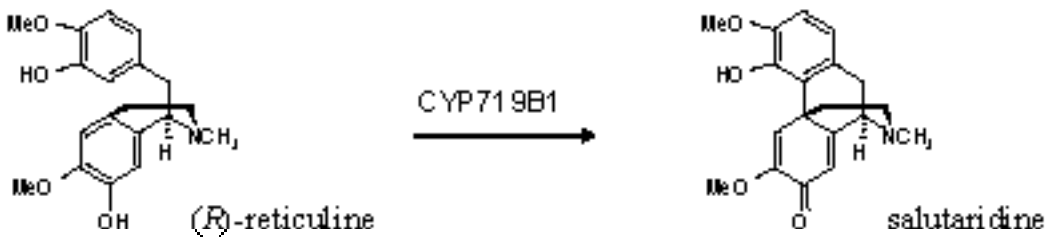
Cytochrome P450s catalyze a wide array of oxidative reactions in secondary metabolism. Using high alkaloid producing cell cultures or cell cultures with induced alkaloid biosynthesis, three cytochrome P450 subfamilies from isoquinoline biosynthesis were identified so far. These subfamilies catalyze a C-O phenol-coupling reaction (CYP80A) in bisbenzylisoquinoline- , a hydroxylation (CYP80B) in benzylisoquinoline- and methylenedioxy bridge formation (CYP719A) in berberine and benzo[*c*]phenanthridine biosynthesis.

We identified salutaridine synthase while selecting for interesting CYP450 cDNAs using sequence comparison, comparative expression profiling of morphine-producing and non-producing plants and tests of transcript accumulation after elicitation of alkaloid biosynthesis in *Papaver somniferum* cell cultures. The (*R*)-reticuline conversion to salutaridine was achieved after protein over-expression in *Spodoptera frugiperda* cells and verified by LC-MS and LC/MS-MS.



PNP-9 (Graduate Student)

ENDOGENOUS MORPHINE IN MAMMALS

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Morphine, one of the strongest analgesic compounds known and a major compound in the plant *Papaver somniferum*, has been found to be present in 10 nM concentration in mammals. The question as to whether it is of endogenous biosynthetic or dietary origin has been clarified by biosynthetic studies done in our lab. We showed unequivocally for the first time that morphine is synthesized in mammalian tissue such as neuroblastoma and pancreas carcinoma cells by performing incorporation experiments with $^{18}O_2$ and feeding experiments involving heavy isotope-labeled precursors. With these results we developed a putative pathway for the biosynthesis of endogenous morphine in mammals and compared this pathway with that in the poppy plant. Enzymatic studies with porcine, rat and mouse liver microsomes as well as human cells and enzymes seem to confirm the proposed biosynthetic pathway and revealed first clues as to the enzymatic mechanisms involved in the formation of endogenous morphine in mammals.

PNP-10 (Graduate Student)

SCREENING *CHRYSOTHAMNUS NAUSEOSUS* (RABBIT BRUSH) POPULATIONS FOR VARIATION IN RUBBER CONTENT AND QUALITY

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Chrysothamnus nauseosus, Rabbit brush, has been known to produce high quality rubber with yields ranging from 1.5% to 6.5% of shoot dry weight. Since Rabbit brush thrives in marginal soils and under drought conditions, it is an ideal crop for lands currently considered as non-arable.

Preliminary studies were performed to determine the natural variation in rubber content and quality produced by wild strands of Rabbit brush. Samples were collected from fourteen different sites in Nevada and California. From these studies high rubber producing sites were identified for further investigation. Rabbit brush samples from four different sites were used to study the rubber production and quality throughout the summer season. Samples were collected from the same plants every two weeks for about five months. The variation of rubber content and the quality was compared to environmental factors such as temperature and precipitation during this period of time in the designated areas. Furthermore, whole plants were collected at the beginning and the end of the collection period from each location. Seeds from each plant were collected at the end of the collection period for future experiments.

The washed rubber particle proteins from Rabbit brush will be characterized and compared to Russian Dandelion (*Taraxacum kok-saghyz*) and Guayule (*Parthenium argentatum*), other members of the Compositae family.

Overall goals of this project are to improve rubber quality and quantity in Rabbit brush and introduce it to the United States as a good economical alternative source of rubber.

PNP-11 (Graduate Student)

INDIVIDUAL CORRELATION OF ARTEMISININ CONTENT IN GAMMA-IRRADIATED PLANTLETS AND THEIR *EX VITRO* PLANTS OF *ARTEMISIA ANNUA*

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One thousand shoot tips obtained from *in vitro* plantlets of *Artemisia annua* were exposed with gamma rays at a dose-range from 100 to 1,000 rad and transferred to hormone-free MS medium for plantlet development. Estimation of the survival percentage of each dose treatment indicated that the LD_{50} value was at the dose of 800 rad. With this treatment, those plantlets surviving after subsequent four subculture passages appeared to have a high variation of artemisinin content, ranging from 0.02 to 0.70 % (w/w) of dry weight. Many of these irradiated plantlets showed a good correlation between the enzyme activity of amorpha-4,11-diene synthase (ADS), the first enzyme of the artemisinine biosynthetic pathway, suggesting that gamma irradiation has significant effect on the ADS gene which might be related to enhancement of artemisinin content in *A. annua*. When some selected plantlets were transferred from the *in vitro* culture to *ex vitro* conditions of greenhouse or open-field, it was found that the mature plants also contained artemisinin in a similar content pattern, interestingly, with a direct correlation of each plantlet-plant pair. These results suggested that stable high artemisinin-yielding plants of *A. annua* can be obtained by the technique of gamma irradiation.

PNP-12 (Graduate Student)

COMPARATIVE GENOMICS OF TRANSCRIPTIONAL CONTROL OF PLANT SPECIALIZED METABOLISM

Eric McDowell, Jeremy Kapteyn and David R. Gang (The University of Arizona)

Plants produce a large array of specialized compounds, such as alkaloids, terpenes, and phenylpropanoids, which are involved in plant defense, herbivory protection and pollen dispersal. Considerable variation, even within the same species, has been observed for the number and types of specialized compounds produced. The aromatic herb sweet basil (*Ocimum basilicum*) is one such plant possessing chemotypes with distinctive chemical profiles. Basil chemotypes are differentiated based on the aromatic and medicinal compounds produced in specialized structures on the surface of the leaves known as peltate glandular trichomes. Digital gene expression analysis of chemotype-specific, peltate glandular trichome EST databases revealed that a chemotype's transcriptional profile (i.e., the necessary enzymatic components of the desired pathway) corresponds reasonably well to what one would expect for its respective metabolic profile. Moreover, the transcription of other, non-enzymatic, proteins such as transcription factors also appears to be differentially controlled between chemotypes, indicating a possible mechanism to explain some of the metabolic differences between chemotypes. We used a comparative genomics approach to identify 13 putative transcription factors with significant differential expression in peltate glandular trichomes between chemotypes. We are currently in the process of cloning full length cDNAs of these transcripts to be used for subsequent functional characterization, which will be discussed.

PNP-13 (Graduate Student)

INDUCTION OF STILBENE FORMATION IN HAIRY ROOTS OF *NICOTIANA BENTHAMIANA*

Cesar Nopo-Olazabal, Luis Nopo-Olazabal, Ganapathy Sivakumar, Jose Condori, Robyn Hannigan and Fabricio Medina-Bolivar (Arkansas Biosciences Institute, Arkansas State University)

Stilbenes are phenolic compounds found in a selected group of taxonomically unrelated plant species such as peanuts and grapes. Among the stilbenes, resveratrol and its derivatives have been associated with important health benefits including antioxidant, anticancer, and anti-aging properties. Because of these health benefits, it is important to study the biosynthetic potential of plants for resveratrol. We have recently demonstrated that peanut hairy root cultures can be induced to produce and secrete resveratrol and derivatives upon exposure to sodium acetate (Medina-Bolivar et al., Phytochemistry, *in press*). In order to investigate the biosynthetic potential for resveratrol in other plant families, we developed hairy root cultures of different species of the Solanaceae. Preliminary screening for resveratrol by HPTLC suggested that hairy roots of *Nicotiana benthamiana* respond to sodium acetate by induction and secretion of putative stilbenoid compounds. To confirm these observations, four hairy root lines of *N. benthamiana* were elicited for 24 hours with sodium acetate and the ethyl acetate extract of the culture medium was analyzed by HPTLC, HPLC with coupled PDA and fluorescence detection and HPLC-MS. The results indicated that these hairy root cultures produce and secrete stilbenes, being the first report of these compounds in the genus *Nicotiana*. Our results suggest that the biosynthesis of stilbenoid compounds could be induced in some species not previously shown to produce these compounds. Furthermore, we highlight the value of the hairy root technology for the discovery of novel bioactive compounds.

PNP-14 (Graduate Student)

TOWARDS UNDERSTANDING THE PHYSIOLOGICAL ROLES OF LACCASES IN *ARABIDOPSIS THALIANA* AND *TELLIMA GRANDIFLORA*

Turlapati V. Phanikanth, Michaël Jourdes, Laurence B. Davin, Norman G. Lewis (Institute of Biological Chemistry, Washington State University, Pullman, WA-99164-6340, USA)

Laccases were first discovered in the Japanese lacquer tree (*Rhus vernicifera*); since the nineteen-fifties, they were also implicated in lignification. None of the studies unequivocally established any true physiological role of laccases in lignification, or any other biochemical process–except for one report of a specific laccase isoform being involved in *Arabidopsis* seed coat formation. This study was aimed at understanding the physiological role of laccases using *Arabidopsis* and *Tellima grandiflora*. In this respect, characterization of the spatial and temporal expression patterns of the 17-membered laccase gene family member in *Arabidopsis* was carried out, using the GUS-reporter system. These studies indicated partially overlapping expression patterns in both lignified/non-lignified tissues, but with no identification of precise physiological role(s). To begin to gain insight into biochemical/physiological roles, available single *Arabidopsis* knockouts in laccase genes were screened for phenotypic and genotypic changes; except for AtLAC14, no other mutant lines showed significant changes in phenotypic appearances. The AtLAC14 mutant has a pale yellow seed coat, indicating a probable function in the condensed tannin pathway. We have thus expressed AtLAC14 in recombinant form to characterize it biochemically. Laccases are also apparently involved in ellagitannin biosynthesis in *Tellima grandiflora*. To further understand their roles in this species, a laccase from leaf tissues was first cloned by degenerate PCR, expressed in recombinant form, and is currently being characterized. Isolation of other laccases from tissues that generate cornusin and tellimagrandin, are also underway. The progress made thus far in determining physiological functions of laccases is described.

PNP-15 (Graduate Student)

BIOSYNTHESIS OF BIOACTIVE 9,9′-DEOXYGENATED LIGNANS, NORDIHYDROGUAIARETIC ACID AND CONOCARPAN

Daniel G. Vassão[1], Sung-Jin Kim[1], Joaquim V. Marques[2], Jessica K. Milhollan[1], Dietmar Eichinger[1], Massuo J. Kato[2], Laurence B. Davin[1] and Norman G. Lewis[1] ([1]Washington State University, [2]University of São Paulo)

9,9′-deoxygenated lignans have beneficial medicinal properties, including nordihydroguaiaretic acid (NDGA) from the creosote bush (*Larrea tridentata*), whose tetramethyl derivative (M4N) is currently in Phase 2 trials for treatment of brain and CNS tumors; others include the insecticidal/antimicrobial (+)-conocarpan in *Piper regnellii*. Their biosynthesis can be envisaged to utilize allyl/propenyl phenol monomers, which could undergo either regio- and/or stereo-selective coupling to afford various lignan skeletal types. Monomeric allyl/propenyl phenols also have important flavor/fragrance and antimicrobial properties.

In our studies, allyl/propenyl phenol biosynthesis was initially investigated in basil (*Ocimum basilicum*), and has since also been extended to both species. In all cases, hydroxycinnamyl alcohol esters served as substrates for regiospecific NAD(P)H-dependent reduction to form allyl/propenyl phenols, via action of chavicol/eugenol or *p*-anol/isoeugenol synthases.

Based on homology to known PIP reductases, 2 genes were isolated from the creosote bush (LtCES1 and 2) and 3 genes from *P. regnellii* (PrCES1, 2a and 2b). LtCES1 was at least 100-fold more efficient than the previously reported enzymes in basil. The roles of allyl/propenyl phenols as stereoselective coupling substrates to form (+)-conocarpan are now being investigated, as well as how the dehydrogenated derivative, eupomatenoid-6, is formed. Radioactive substrate *in vivo* administration has shown that youngest leaves are most active in lignan biosynthesis, and *in vitro* experiments indicate that 8–3′ coupling uses *p*-anol (and not chavicol) as substrate. Further characterization of the proteins involved, as well as that of the conocarpan dehydrogenation will be described.

PNP-16 (Graduate Student)

DITERPENE RESIN BIOSYNTHESIS IN *PINUS DENSIFLORA* IS REGULATED BY TWO OF THE METHYLERYTHRITOL PHOSPHATE PATHWAY GENES, *DXS* AND *IDS*

Yeon-Bok Kim[1], Sang-Min Kim[1],Yung Jin Chang[1] and Soo-Un Kim[1,2] ([1]Seoul National University and [2] Plant Metabolism Research Center, Kyunghee University)

Pinus densiflora is endemic to the Korean peninsular to constitute a major green canopy in the mountainous area. We cloned cDNAs of 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), and isopentenyl diphosphate/dimethylallyl diphosphate synthases (IDS) involved in methylerythritol phosphate (MEP) pathway, which supplies building blocks for the resin biosynthesis, using rapid amplification of cDNA ends (RACE) technique. In addition, abietadiene synthase (ABS) was cloned as a marker for resin biosynthesis. Surprisingly, *PdIDS* appeared as a two-gene family in addition to *PdDXS. PdDXSs*, *PdDXR*, and *PdIDS*s formed a separate clade from the angiosperm genes in phylogenetic trees. Transcription levels of *PdDXS1, PdDXR,* and *PdIDS1* were comparable in all tissues, while *PdDXS2* and *PdIDS2* were most actively transcribed in stem wood where high transcription level of *PdABS* appeared. Thus, we suggest that *PdDXS2* and *PdIDS2* have high correlation with resin biosynthesis in the pine.

PNP-17 (Graduate Student)

CHIMERIC ANALYSIS OF CLASS II DITERPENE SYNTHASES

Sladjana Prisic, Francis Mann, and Reuben J. Peters (Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA)

Terpene synthases are responsible for the stereospecific rearrangement and/or cyclization of linear isoprenoid substrates into complex hydrocarbon skeletal structures, using electrophilic reaction mechanisms initiated by carbocation formation. The initial carbocation can be generated by either ionization of the allylic pyrophosphate (class I enzymes) or protonation of an epoxide ring or carbon-carbon double bond (class II enzymes). Prototypical class II diterpene synthases catalyze the production of copalyl diphosphate (CPP) from geranylgeranyl diphosphate (GGPP), and are generally termed Copalyl Diphosphate Synthases (CPS). Rice (*oryza sativa*) contains three such enzymes (OsCPS) with distinct product stereospecificity and metabolic roles: OsCPS1, and OsCPS2, which produce *ent*-CPP for gibberellin and phytoalexin production, respectively, and OsCPS4, which produces *syn*-CPP for phytoalexin and allelochemical biosynthesis. All of these enzymes contain the three structural domains typical of class II diterpene synthases (i.e. insertional, N-terminal, and C-terminal domains), but exhibit only ~51% sequence identity. Six chimeras of OsCPS2 and OsCPS4 were constructed using domain-swapping techniques. Of these domains, the N-terminal domain contains the catalytic DXDD motif, but all three domains are required for proper folding and enzymatic activity. Swapping of the C-terminal domain lead to stereospecific conversion of GGPP into *ent*- or *syn*-CPP corresponding to the origin of the insertional and N-terminal domains. Preliminary results further suggest that swapping of the insertional domain results in the stereospecific CPP product corresponding to the origin of that domain. These results indicate a dominant role for the insertional domain in determining product specificity for class II diterpene synthases.

PNP-18 (Graduate Student)

CLARIFYING RICE GIBBERELLIC ACID METABOLISM

Qiang Wang and Reuben J. Peters (Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University)

Extended enzymatic families related to those typically involved in GA metabolism have been identified in the rice genome. However, recent results emphasize the extent to which even GA metabolism remains unexplored. In particular, two novel classes of catabolic enzymes have been recently identified. The first are 2-oxoglutarate dependent dioxygenases (2ODDs) catalyzing hydroxylation at the C2 position of 20 carbon GAs (e.g. GA_{12}) from dicot species. Although 2ODD GA2-oxidases (GA2ox) have been previously identified, these are specific for the 19 carbon nor-diterpenoid forms of GA. The second recently identified GA catabolic enzyme is a P450 (CYP714D1) catalyzing epoxidation of the 16,17-diene. We have cloned some homologs of the alternative GA2ox in rice and are in the process of characterizing their enzymatic activity after heterologous expression in *E.coli*. Preliminary results suggest that these putative GA2ox catalyzed hydroxylation of GA12 (20 carbon gibberellin).Verification of the expected enzymatic activity awaits authentic standards for the presumed GA110 product. The rice genome contains a small family of homologs to the recently identifed catabolic CYP714D1, which we are in the process of cloning for recombinant expression in the WAT11 strain of yeast. Assays with recombinant yeast cells and microsomal preparations will be carried out to determine the biochemical activity. Our most current result in this project will be presented.

PNP-19 (Graduate Student)

INVESTIGATIONS OF SUBSTRATE AND PRODUCT SPECIFICITY IN CLASS I DITERPENE SYNTHASES

Ke Zhou and Reuben J. Peters (Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University)

Diterpene synthases catalyze arguably the most complex chemical reactions occurring in Nature, and are of significant mechanistic interest. We are investigating substrate and product specificity through comparative homology modeling of class I labdane-related diterpene synthases with different copalyl diphosphate (CPP) substrate stereospecificity or product outcome. For example, abietadiene synthase (AS) specifically recognizes normal CPP and its class I active site is shaped, in part, by a protruding Tyr residue underneath the DDXXD motif and an opposing Ile across the cavity, both of which are conserved in other normal CPP specific diterpene synthases. In contrast, kaurene synthases (KS) specifically utilize *ent*-CPP, and contain a Met or Leu in place of the Tyr, along with an opposing conserved Phe rather than Ile. Therefore, these residues were reciprocally mutated in AS and KS, and the resulting mutant enzymes will be examined for changes in stereoselectivity as well as altered kinetic parameters. In addition to substrate specificity we also are interested in the determinants of product outcome in diterpene synthases. Previous work determined that substitution of a conserved isoleucine to threonine on the 'F' helix is sufficient to essentially switch the catalytic specificity of a wide range of (iso)kaurene synthases to the production of pimaradiene. We now further report that a nearby valine/leucine residue on the 'F' helix also seems to be involved in product specificity as we found that increasing side chain volume decreases the product specificity of rice isokaurene synthases, leading to increased production of atiserene and kaurene. These results highlight the importance of the 'F' helix to product outcome, and progress towards more comprehensive analysis of the corresponding residues will be presented.

PNP-20 (Postdoctoral)

STRUCTURAL STUDY OF ALLENE OXIDE SYNTHASE INVOLVED IN JASMONIC ACID BIOSYNTHESIS

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Crystallization and structural determination of eukaryotic cytochrome P450s are challenging because of the bottleneck to generate soluble, homogeneous P450 protein samples for growing diffraction quality crystals. So far, several crystal structures of mammalian microsomal P450s (P450 2C5, 2B4, 2C8, 2C9, 2A6, 3A4 and 2D6) have been determined. There are large numbers of cytochrome P450s present in the plant kingdom; however, no plant P450 structure has been reported yet. Allene oxide synthase (AOS) is a key enzyme in the biosynthesis of jasmonates. AOS enzymes are members of subfamily CYP74 of the cytochrome P450 superfamily. AOS from *Parthenium argentatum* (PaAOS) has 65% amino acid identity and 85% similarity with a flaxseed AOS. It metabolizes hydroperoxide substrates, 13S-HPODE, 13S-HPOTE and 15S-HPETE, and was assigned as CYP74A2. Amino acid sequence alignment indicated PaAOS contains four common domains of the cytochrome P450 enzyme, but does not have the signal peptide for chloroplast targeting; thus, PaAOS is an ideal target for crystallization of the first plant cytochrome P450. PaAOS gene was cloned into an expression vector pET28a for overexpression in *E. coli* and purification of its His_6-tagged form for crystallographic study. Crystals have been obtained and diffracted to 2.5 Å resolution. Structure analysis is in progress. Some preliminary results will be presented. This study will provide a structural basis for understanding the enzymatic mechanism and substrate specificity of PaAOS as well as the structure-function relationship of plant cytochrome P450s.

PNP-21 (Postdoctoral)

ALLYLIC DOUBLE BOND REDUCTASES IN *ARABIDOPSIS* AND *PINUS* SPECIES

Sung-Jin Kim[1], Choonseok Lee[1], Ann M. Patten[1], Diana L. Bedgar[1], Syed G.A. Moinuddin[1], Buhyun Youn[2], ChulHee Kang[2], Laurence B. Davin[1] and Norman G. Lewis[1] ([1]Institute of Biological Chemistry and [2] School of Molecular Biosciences, Washington State University, Pullman, WA)

Gymnosperms biosynthesize various phenylpropanoid derivatives whose 7–8 allylic double bonds are reduced; these compounds are formed, for example, as an inducible response to aphid (*Adelges abietis*). The corresponding phenylpropanoid double bond reductase (PPDBR) was isolated from loblolly pine (*Pinus taeda*), and demonstrated to reduce substrates, such as coniferyl/ dehydrodiconiferyl aldehydes, to their corresponding dihydro derivatives. Using *in situ* mRNA hybridization, it was also established that PPDBR mRNA was expressed in the vascular cambium and radical/axial parenchyma cells – in apparent agreement with a plant defense function.

Based on gene sequence comparison (data mining), a putative 11-membered alkenal double bond reductase family was found in *Arabidopsis*. All 11 genes were cloned, with recombinant proteins obtained using *E. coli* as an expression system. Only three were demonstrated able to reduce coniferyl/dehydrodiconiferyl aldehydes as potential substrates; the closest homologue (AtDBR1, *At5g16970*) was also able to reduce 4-hydroxy-(2*E*)-nonenal, a toxic substance to mammals. These enzymes belong to the zinc-independent, medium chain dehydrogenase/reductase superfamily, and exist in dimeric form. Crystal structure determination of *apo*- , binary and ternary complexes, has established their potential binding/catalysis residues; each of these was next mutated, with the mutant enzymes comprehensively biochemically analyzed to probe further the catalytic mechanism envisaged. Patterns of gene expression for each of the 11 recombinant forms were also determined [i.e. as a first step towards establishing physiological function(s)]; interestingly, AtDBR1 was strongly expressed in wounded tissues – in agreement with its proposed role in plant defense.

PNP-22 (Postdoctoral)

REGULATORY PROTEIN-PROTEIN INTERACTIONS IN PLANT CYSTEINE BIOSYNTHESIS

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Cysteine biosynthesis is catalyzed by /O/-acetylserine sulfhydrylase (OASS). However, during high sulfur flux, OASS interacts with the C-terminus of serine acetyltransferase (SAT) to form cysteine synthase, which acts as a molecular sensor to regulate cysteine production. We have studied the protein-protein interactions in the complex using isothermal titration calorimetry (ITC), sedimentation equilibrium (SE), and surface plasma resonance (SPR) techniques. Our sedimentation and gel filtration studies show that soybean SAT is a homotrimer in contrast to bacterial SAT, which exists as homohexamer. ITC and SE studies show that three OASS dimers interact with a single SAT trimer to form a complex with a total molecular weight of ~330 kDa. Both SPR and ITC indicate that OASS dimers interact to SAT with very high affinity (~0.5 nM). The kinetics of complex formation reveal that dissociation of the SAT-OASS complex is very slow and that the complex cannot be completely dissociated passively. These findings are consistent with structural studies showing that the C-terminal peptide of SAT occupies the *O*-acetylserine (OAS) binding site in OASS. Thus, dissociation of the OASS-SAT complex could be facilitated through OAS binding. We propose that formation and dissociation of the complex use two different mechanisms. Interaction of OASS with SAT to form cysteine synthase is both thermodynamically and kinetically favored, but the breaking of complex involves an active mode of dissociation that may require the binding free energy of OAS to OASS. Evolution of passive association and active dissociation between SAT and OASS results in a molecular circuit for controlling sulfur assimilation and cysteine biosynthesis in plants.

PNP-23 (Postdoctoral)

BIOSYNTHESIS OF DEFENSIVE SECONDARY METABOLITES, BENZOXAZINONES, IN POLYPLOID WHEAT

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Benzoxazinones (Bxs), e.g. DIBOA and DIMBOA, are representative defensive secondary metabolites in hexaploid wheat (*Triticum aestivum*, 2n=6x=42, genomes AABBDD). Five Bx biosynthetic genes, *TaBx1-TaBx5*, are located on each of the three genomes (A, B and D) of hexaploid wheat. To reveal how each genome contributes to the Bx biosynthesis in a complex hexaploid genome, we compared transcript levels and enzyme activities of the *TaBx* genes among the three genomes. The three homoeologs were found to be differentially transcribed, and the translation products of the three homoeologs had different catalytic properties. Considering the transcript levels and the catalytic properties collectively, we concluded that the biosynthetic genes on the B genome generally contribute the most to Bx biosynthesis in hexaploid wheat. To reveal the mechanism of differential transcription of the *TaBx* genes among the three genomes of hexaploid wheat, promoter sequences of the three homoeologs of *TaBx3* and *TaBx4* were isolated and promoter activity measured by the transient assay in wheat protoplasts using luciferase as reporter. In spite of the differential transcript levels among the three homoeologs of *TaBx3* and *TaBx4*, the promoter activity was similar among the three homoeologs. This result suggests that the differential transcription among the three homoeologs does not result from the differences in promoter activity. We propose that epigenetic gene regulation is involved in the differential transcription of the three *TaBx* homoeologs in hexaploid wheat.

PNP-24 (Postdoctoral)

A CYTOCHROME P450 ENZYME FROM *ILLICIUM PARVIFLORUM* CAPABLE OF CATALYZING THE FORMATION OF A METHYLENEDIOXY BRIDGE ON EUGENOL TO PRODUCE SAFROLE

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Phenylpropanoids constitute a major group of secondary compounds present in the plant kingdom. The volatile phenylpropenes, a subgroup of the phenylpropanoids in which the propyl side-chain is reduced, include the well-known spice eugenol as well as safrole, myristicin, apiol, and dillapiole; they are also believed to function as defense toxins against herbivores and pathogens as well as in attracting pollinators. Such compounds have been found in basil, parsley, dill, yellow anisetree and many other plant species dispersed throughout angiosperm taxa, although in many cases closely related species do not make the same set of phenylpropene compounds, at least not in detectable levels. Here we report a putative *safrole synthase gene*, isolated from *Illicium parviflorum* (yellow anisetree), encoding a cytochrome P450 enzyme that is capable of converting eugenol to safrole via the formation of a methylendioxy bridge. High levels of safrole are detected in both leaves and flowers of *I. parviflorum* and lower levels are present in the stem. Transgenic yeast cell cultures and *Arabidopsis* plants overexpressing the *safrole synthase gene* and fed with eugenol are able to convert it to safrole. *In vitro* enzyme assay with a microsomal fraction of *Arabidopsis* plantlets shows detectable activity. In addition, while petunia flowers normally emit high levels of isoeugenol and very low levels of eugenol, but no safrole or isosafrole, transgenic petunia flowers transformed with the *safrole synthase gene* emit low levels of isosafrole in addition to isoeugenol and eugenol.

PNP-25 (Postdoctoral)

IDENTIFICATION AND CHARACTERIZATION OF AROGENATE DEHYDRATASE(S) IN *ARABIDOPSIS*: COMPARISON TO A BACTERIAL PREPHENATE DEHYDRATASE

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While both prephenate and arogenate have been reported in plants to undergo decarboxylative dehydration via the action of dehydratases to afford phenylpyruvate and phenylalanine, respectively, neither enzyme(s) nor encoding gene(s) were isolated and/or identified. In this study, a data mining approach was undertaken to attempt to identify the dehydratase(s) involved in Phe formation. This approach suggested that there were six putative prephenate dehydratase (PDT) homologues in *Arabidopsis*, based on similarity to bacterial PDTs. However, earlier biochemical analyses of cell extracts detected arogenate dehydratase (ADT) rather than PDT activities. All six putative ADTs/ PDTs were cloned and expressed as Nus-tagged recombinant proteins in *E. coli*. Three of the resulting recombinant proteins more efficiently utilized arogenate than prephenate, with k_{cat}/K_m values of 7650 and 1300 M^{-1} s^{-1} for arogenate, vs. 38, 240 and 16 M^{-1} s^{-1} for prephenate, respectively. The remaining three, by contrast, had k_{cat}/K_m values of 414, 265 and 370 M^{-1} s^{-1} for arogenate, with prephenate not serving as a substrate unless excess recombinant protein (>150 μg/assay) was used. For comparative purposes, a previously characterized PDT from *Methanocaldococcus jannaschii* was assayed under the same conditions; it was shown to have a very strong substrate preference for prephenate over arogenate. All six *Arabidopsis* genes, and their corresponding proteins, are thus provisionally classified as arogenate dehydratases and designated ADT1 through ADT6.

PNP-26 (Postdoctoral)

A RICE CYTOCHROME P450 KAURENE OXIDASE HOMOLOG WITH A POTENTIAL ROLE IN LABDANE- RELATED DITERPENOID PHYTOALEXIN BIOSYNTHESIS

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Rice, *Oryza sativa,* is a staple food crop and produces more than ten related diterpenoid natural products that act as phyotalexins, exhibiting antibiotic activity against the devastating blast fungal pathogen, *Magneporthe grisea*. Cytochromes p450 are necessary for the enzymatic oxidation of olefin diterpene precursors to produce bioactive phytoalexins. For example, rice has five putative members of the CYP701A family, only one of which functions as the ubiquitous kaurene oxidase (OsKO2) in gibberellin production. The functions of the remaining members of this gene family in rice are unknown. We hypothesize that these are involved in diterpenoid phytoalexin biosynthesis. In the present investigation, one such P450 i.e *Oryza sativa* Kaurene Oxidase like P450 enzyme 4 (OsKOL4) was cloned and expressed in insect cells using a baculovirus based expression system. Functional characterization of this recombinant P450 is underway. Functional characterization of the P450 enzymes involved in rice diterpenoid metabolism will provide a model system for investigating the underlying structure-function relationships and further provide a foundation for future studies utilizing these enzymatic genes in metabolic engineering efforts for the production of specific individual terpenoid ‘natural’ products.

PNP-27

SUBERIN BIOSYNTHESIS: A METABOLOMICS APPROACH

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Suberin is a specific cell wall-associated biopolymer characterized by the deposition of both a poly(phenolic) domain (SPPD) associated with the cell wall, and a poly(aliphatic) domain (SPAD) thought to be deposited between the cell wall and plasma membrane. *In planta*, suberin functions to protect plants from desiccation and pathogen attack. Although the chemical identity of the monomeric components of the SPPD and SPAD are well known, their concerted biosynthesis and assembly into the suberin macromolecule is poorly understood. To expand our knowledge of suberin biosynthesis, a GC/MS-based metabolite profiling study was conducted, using wound healing potato (*Solanum tuberosum* L.) tubers as a model system. A time series of both non-polar and polar metabolite profiles were created, yielding a broad-based, dynamic picture of wound-induced metabolism, including suberization. Principal component analysis revealed a separation of metabolite profiles according to different suberization stages, with clear temporal differences emerging in the non-polar and polar profiles. In the non-polar profiles, suberin-associated aliphatics contributed the most to cluster formation, while a broader range of metabolites (including organic acids, sugars, amino acids and phenylpropanoids) influenced cluster formation amongst polar profiles. Pair-wise correlation analysis revealed strong correlations between known suberin-associated compounds, as well as between suberin-associated compounds and several un-identified metabolites in the profiles. These data may help to identify additional, as yet unknown metabolites associated with suberization process.

PNP-28

IN VITRO PLANT, CALLUS AND ROOT CULTURES OF *PLUMBAGO INDICA* AND THEIR BIOSYNTHETIC POTENTIAL OF PLUMBAGIN

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In vitro cultured plants of *Plumbago indica* L. were established from nodal segments and micropropagated on hormone-free LS medium. These *in vitro* plantlets produced plumbagin with the content 0.79-0.87 mg g^{-1} dry weight, which was more than half of the content found in the whole roots of greenhouse plants. Root and callus cultures were also initiated from stem and young leaf explants, respectively. The root cultures maintained in hormone-free MS medium accumulated 0.28 mg g^{-1} plumbagin whereas the callus cultures grown on MS medium supplemented with 1.0 mg l^{-1} 2,4-dichloropenoxyacetic acid (2,4-D) and 0.1 mg l^{-1} kinetin contained only 0.013 mg g^{-1} of the compound. In addition to plumbagin, its related compounds plumbagic acid and plumbagic acid glucoside were also found specifically in the root tissues of the micropropagated plantlets and the root cultures. These results suggested the biosynthetic potential for the plumbagin-derived compounds in the tissues of *in vitro* plants and organ cultures, which allows us to use them as materials for studying genes and enzymes involved in the naphthoquinone formation in *P. indica*.

PNP-29

JUSTICIDIN B 7-HYDROXYLASE, A CYTOCHROME P450 MONOOXYGENASE FROM CELL CULTURES OF *LINUM PERENNE* HIMMELSZELT INVOLVED IN THE BIOSYNTHESIS OF DIPHYLLIN

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Cell suspension cultures of *Linum perenne* Himmelszelt accumulate the lignan justicidin B as the main component together with glycosides of 7-hydroxyjusticidin B (diphyllin). Justicidin B 7-hydroxylase (JusB7H) was characterized from a microsomal fraction prepared from *Linum perenne* Himmelszelt suspension culture for the first time. The enzyme catalyzes the introduction of the hydroxyl group in position 7 of justicidin B and results in the formation of diphyllin. The hydroxylase activity was strongly inhibited by cytochrome *c* as well as other cytochrome P450 inhibitors such as clotrimazol, indicating the involvement of a cytochrome P450 enzyme. JusB7H has a pH optimum of 7.4 and a temperature optimum of 26 °C. Justicidin B was the only accepted substrate by JusB7H with an apparent K_m of 3.9 µM and a saturation concentration of 50 µM. NADPH is accepted predominantly as the electron donor by JusB7H. NADH can only sustain very low hydroxylation activities. A synergistic effect of NADPH and NADH was not observed. The apparent K_m for NADPH as cosubstrate is 102 µM.

PNP-30

THE EVOLUTION OF PINORESINOL-LARICIRESINOL REDUCTASES

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Lignans of a wide variety of structural types and enantiomeric forms are found in the plant kingdom. Despite this large diversity, the first biosynthetic steps seem to be the same in different species. Two molecules of coniferyl alcohol are dimerised to pinoresinol (Pino) which is further reduced via lariciresinol (Lari) to secoisolariciresinol (Seco) by the pinoresinol-lariciresinol reductase (PLR). In cell cultures of *Linum album* and *L. usitatissimum*, the enantiomeric purity is determined by the enantiospecificity of the PLR. In both cultures Pino is a mixture of both enantiomers, the Seco in *L. album* has R,R-, that of *L. usitatissimum* S,S-configuration. We are interested in understanding the evolutionary background for these differences in enantiospecificity. Therefore, we cloned PLRs and related enzymes from *Arabidopsis thaliana* and *Linum* species accumulating different types of lignans. The functionality of the recombinant proteins was distinguished by using dehydrodiconiferyl alcohol (the substrate for PCBERs) and racemic Pino as substrates. The enantiospecificity of the PLRs was determined by chiral column chromatography of the remaining Pino and formed Lari and Seco from reactions with different protein concentrations or after different reaction times. We found a broad variation in enantiospecifity. We figured out which amino acids in the PLRs are responsible for the different enantiospecificity by a mutagenesis approach.

Acknowledgements: We thank the DFG (SPP1152, Evolution of Metabolic Diversity) for financial support.

PNP-31

MODULATION OF FREE AMINO ACIDS AND GAMMA-GLUTAMYL DIPEPTIDES IN RESPONSE TO STORAGE PROTEIN DEFICIENCY IN SEEDS OF COMMON BEAN

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A study was conducted to characterize the impact of storage protein deficiency on the accumulation of seed reserves in white navy bean (*Phaseolus vulgaris*). Three genetic lines combine deficiencies in the major storage proteins, phaseolin (Phs), phytohemagglutinin (Lec) and arcelin (Arl) (Osborn et al. 2003 Crop Sci. 43, 1570). The three lines, SARC1, SMARC1-PN1 (*phs*) and SMARC1N-PN1 (*phs*, *lec*, *arl*), share a common genetic background from the commercial cultivar Sanilac. Genotypes were initially confirmed by SDS-PAGE. Overall, the three lines had similar carbon, nitrogen and sulfur, and total extractible protein content. However, storage protein deficiency was significantly correlated with higher levels of cytoplasmic proteins. Storage protein deficiency was partially compensated by a progressive increase in total free amino acids (by 2-fold in SMARC1N-PN1 as compared with SARC1). Most individual free amino acids were elevated, especially arginine (3.8-fold) and asparagine (1.6-fold), as well as the dipeptide □-glutamyl-leucine (1.8-fold). By contrast, □-glutamyl-*S*-methyl-cysteine and *S*-methyl-cysteine were decreased by 1.8-and 1.3-fold, respectively. These results suggest that protein acts as a sink for sulfur in the storage protein deficient lines, and that □-glutamyl-*S*-methyl-cysteine constitutes a form of sulfur storage in seeds of common bean.

PNP-32

PHYTOTOXICITY OF TRICHOTHECENES USING AN *ARABIDOPSIS* DETACHED LEAF ASSAY

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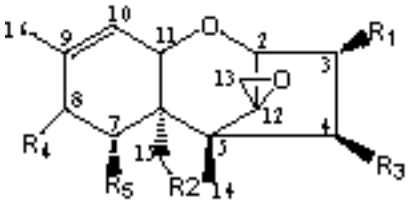
Trichothecenes are sesquiterpenoid epoxide mycotoxins produced by *Fusarium* and other fungi. Although some *Fusarium* trichothecenes are virulence factors in plant disease, the phytotoxicities of many trichothecenes have not been investigated. Results of previous studies, using a limited group of trichothecenes, suggest that trichothecenes with a C-3 acetyl group are less toxic than those with a C-3 hydroxyl group. In order to confirm this finding and to determine if additional structural features might influence phytotoxicity, detached leaves of *Arabidopsis thaliana* were treated with solutions of twenty-four trichothecenes that differed in oxygenation and acetylation and the ED50s were determined. The results confirm that in some, but not all, cases the C-3 acetyl group reduces phytotoxicity, and that other structural features may be important.



PNP-33

MOLECULAR CLONING OF STRICTOSIDINE SYNTHASE FROM *MITRAGYNA SPECIOSA*

Anan Ounaroon[1] and Wanchai De-Eknamkul[2] ([1]Naresuan University and [2]Chulalongkorn University, Thailand)

Mitragyna speciosa (Roxb.) Korth. or Kratom (Rubiaceae) has been used for diminishing fatigue and enhancing tolerance to the hard work under the scorching sun. From about 40 alkaloids extracted from this plant, the major constituents are indole alkaloids such as mitragynine (leaves, opium-like effect), 7-hydroxymitragynine (leaves, antinociceptive effect) and 7-hydroxyspeciociliatine (fruits, opioid agonist). Therefore, *M. speciosa* was selected as a model for the study of the indole alkaloid biosynthesis, particularly the step of the condensation between tryptamine and secologanin catalyzed by strictosidine synthase. The aim of the study is to clone and characterize recombinant *strictosidine synthase* (*SSS*). Total RNA was isolated from fresh leaves and converted to cDNA using SMART-RACE. The degenerated primers for PCR were designed based on the multiple alignment of strictosidine synthase from *Catharanthus roseus*, *Ophiorrhiza pumila* and *Rauvolfia serpentina*. The full-length *SSS* from *M. speciosa*, encoding 352 amino acids, shows 70%, 53% and 52% similarity to *SSS* from *O. pumila*, *R. serpentina* and *C. roseus* respectively.

PNP-34

MULTIPLE SHOOT REGENERATION AND ARTEMISININ PRODUCTION IN *ARTEMISIA ANNUA* L. USING THIDIAZURON

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An efficient method for multiple shoot bud induction and regeneration *in vitro* has been developed with *Artemisia annua* L. leaf and stem explants in various concentrations and combination of plant growth regulators to evaluate the frequency of regeneration. The sources of the explants as well as plant growth regulator in the medium were found to influence multiple shoot induction. The results show that stems cultured on Murashige and Skoog (MS) medium supplemented with 0.1 mg/l thidiazuron (TDZ) gave the highest percentage of shoot formation (100%) and numbers of shoots per explant (57.33±4.09 shoots/explant) after 2 weeks culture. Healthy regenerated shoots were elongated and rooted in MS medium without hormone addition. Artemisinin contents in plants regenerated from stem explants using TDZ 0.1 mg/l (3.36±0.36 μg/mg dry wt.) were 2-fold higher than that observed in normal *in vitro* grown plants of the same age (1.73±0.23 μg/mg dry wt.). This system gives the potential for rapid of induction shoots from stem explants and regeneration of shoots from *A. annua*.

PNP-35

METABOLISM OF FLAVIN NUCLEOTIDES IN PLANTS

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FMN and FAD are phosphorylated derivatives of riboflavin that are synthesized by the enzymes riboflavin kinase (EC 2.7.1.26) and FAD synthetase (EC 2.7.7.2) in the presence of ATP and Mg^{2+}. These flavin nucleotides are the cofactors for scores of enzymes that participate in vital metabolic processes in all organisms. Despite the vital roles of FMN and FAD in metabolism, much remains to be learned about the enzymes that synthesize and hydrolyze these cofactors in plants. Our long-term goal is to uncover how plants maintain intracellular levels of FMN and FAD.

Toward the goal of advancing basic understanding of FMN and FAD homeostasis in plants, we are now investigating riboflavin kinases, FAD synthetases, FMN hydrolases and FAD pyrophosphatases from *Arabidopsis* and pea. Our results suggest that plants contain sequence homologs of riboflavin kinases from bacteria and yeast, and that they contain a novel type of riboflavin kinase in organelles. Our results also show that plant organelles contain FMN hydrolase and FAD pyrophosphatase activities. Here we present our progress in studying enzymes involved in synthesis and hydrolysis of flavin nucleotides in plants.

PNP-36

REGULATORY ROLE OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE IN PLAUNOTOL BIOSYNTHESIS DURING *CROTON STELLATOPILOSUS* LEAF DEVELOPMENT

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Relationships between the diterpenoid plaunotol accumulation and the expression level of genes encoding 1-deoxy-D-xylulose-5-phosphate synthase (DXS), which catalyzes the first committed step of the glyceraldehyde phosphate (GAP)/pyruvate pathway, and geranylgeranyl diphosphate synthase (GGPPS), which is responsible for the formation of geranylgeraniol, a branching step for plaunotol production in *Croton stellatopilosus*, were investigated. Leaves at different developmental stages ranging from shoot as well as 1st, 2nd, 3rd, 4th and 5th rank on the same branch were used for RNA extraction and plaunotol isolation. Competitive PCR analysis with an internal control indicated that mRNA expressions of DXS and GGPPS were detected in all tissues examined, showing higher in the 2nd and 3rd rank of leaves, where a large quantity of plaunotol accumulated. Thus it suggests that activation of the GAP/pyruvate pathway is able to supply the precursor for subsequent plaunotol biosynthesis in *C. stellatopilosus*.

PNP-37

cDNA CLONING AND EXPRESSION PROFILING OF *DXR* GENE ENCODING 1-DEOXY-D-XYLULOSE 5-PHOSPHATE REDUCTOISOMERASE FROM *CROTON STELLATOPILOSUS* OHBA

Tanawan Sirisuntipong[1], Worapan Sitthithaworn[2], Pimpimon Tansakul[1], Wanchai De-Eknamkul[3] and Juraithip Wungsintaweekul[1] ([1]Prince of Songkla University, HatYai, Songkhla, Thailand, [2]Srinakharinwirot University, Ongkarak, Nakornayok, Thailand, and [3]Chulalongkorn University, Bangkok, Thailand)

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; EC 1.1.1.267) is the second enzyme in the deoxyxylulose phosphate (DXP) pathway. It catalyzes the formation of 2*C*-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate in the presence of NADPH and Mg^{2+} or Mn^{2+}. In this study, the *dxr* gene was cloned from cDNA of *Croton stellatopilosus* young leaves (designated as *CSdxr*) by homology based PCR and rapid amplification of cDNA ends (RACE) methods. The results showed that *CS-dxr* contained an open reading frame (ORF) of 1,404 nucleotides encoding a deduced peptide of 468 amino acid residues. Analyzed data of CSDXR indicated that CSDXR carried the chloroplast transit peptide at the N-terminal (position 1-44), and contained a proline-rich region and NADPH binding motif. Alignment of CSDXR shared high homology with more than 76% amino acid identity to other known plant DXRs. Expression pattern analysis indicated that *CSdxr* was strongly expressed in leaves but rarely in stems and roots. CSDXR was found to be associated with isoprenoid biosynthesis via the DXP pathway; however, it did not appear to be the rate-limiting step in plaunotol biosynthesis.

PNP-38

BIOSYNTHESIS OF -SITOSTEROL AND STIGMASTEROL PROCEEDS EXCLUSIVELY VIA MEVALONATE PATHWAY IN THE DISORGANIZED CELL SUSPENSION CULTURES OF CROTON STELLATOPILOSUS

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[1-^{13}C]Glucose and [2-^{13}C]sodium acetate were fed to cell suspension cultures of *Croton stellatopilosus* and cultured under controlled conditions. β-Sitosterol and stigmasterol were isolated and elucidated for their ^{13}C-labeling patterns using quantitative NMR spectroscopy. Analysis of the patterns of ^{13}C-enrichment revealed that the components of all isoprene units of the phytosterols were supplied almost exclusively from the mevalonate pathway. These results were in contrast with the previous study using callus cultures of *C. stellatopilosus*, which showed that the isoprene units in the molecules of β-sitosterol and stigmasterol originated from mixed origins of the deoxyxylulose phosphate and mevalonate pathways. This study demonstrated that a sole operation by only one of the two possible pathways of isoprene formation for *C. stellatopilosus* is supplied dependently upon the type of culture, cell organelles and phytosterol biosynthesis is possible in a very simple and disorganized tissue as this case of the cell suspension cultures of *C. stellatopilosus*.

PSE-1 (Undergraduate Student)

MEASUREMENT OF THIOPHENE DYNAMICS IN THE MARIGOLD RHIZOSPHERE

Philip D. Boes, Jamie L. Yost and Jeffrey D. Weidenhamer (Department of Chemistry, Ashland University, Ashland OH 44805)

It has long been hypothesized that phytotoxic chemicals released by plants into the soil may function as natural herbicides to inhibit the growth of neighboring plants. If this is the case, such chemicals may influence important ecological processes such as nutrient cycling and successional changes in plant communities over time. Recently, it has been proposed that the success of certain invasive plants might be due to toxic root exudates. One of the major barriers to evaluating these ideas has been the difficulty of analyzing the release and dynamics of these chemicals in the field. It has recently been shown that sorbent materials based on polydimethylsiloxane (PDMS) can be used to monitor lipophilic allelochemicals in plant root exudates. In this study, 5 cm lengths of stainless steel wire coated with PDMS tubing were inserted into the root zone of marigold plants in the field for 1-9 weeks. Both 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) and □-terthienyl were detected beneath plants, with the amount and relative proportion of each compound varying by plant and sampling date. The highest amounts of BBT and □-terthienyl were detected at one week, implying that equilibrium is rapidly established between thiophenes in the soil and the fraction sorbed on PDMS. The results also imply that desorption of thiophenes from PDMS occurs despite their high lipophilicity (estimated log K_{ow} for □-terthienyl is 4.98). Ongoing studies are being undertaken to examine other probe designs to measure time-weighted average concentrations of these thiophenes in the rhizosphere, and to use these probes to screen thiophenes exuded by the roots of a variety of *Tagetes* and *Rudbeckia* species.

PSE-2 (Undergraduate Student)

DIFFUSIVE SAMPLING METHODS TO MONITOR ALLELOCHEMICAL DYNAMICS

Rebecca X. Loi, Marissa C. Solar, David S. Wilcox and Jeffrey D. Weidenhamer (Department of Chemistry, Ashland University, Ashland OH 44805)

A significant barrier to testing hypotheses of allelopathic effects between plants has been the general lack of information on the qualitative and quantitative dynamics of allelochemicals in the rhizosphere, as well as their movement into target plants. 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. The overall goal of this project is the development of materials that can trap allelochemicals as they are released in the rhizosphere so that input rates can be estimated. The characteristics of polydimethylsiloxane (PDMS) based materials are being explored to optimize parameters for their use in the greenhouse and field. The sorption and desorption characteristics of PDMS materials for allyl and benzyl isothiocyanate as a function of PDMS surface area and volume have been a specific focus because of the suspected role of these compounds in the putative allelopathic effects of garlic mustard, *Alliaria petiolata.* New probe designs which shield the sorbent reservoir to allow long-term trapping of compounds are being explored. In addition, PDMS on SPME fibers *in vivo* has been used to demonstrate uptake of exogenously applied 8-methoxypsoralen by potted tomato plants.

PSE-3 (Postdoctoral)

SUCCESSIVE PROCESSING OF *ARABIDOPSIS* SEED PROTEINS REVEALED BY PROTEOMIC ANALYSIS

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Seed storage proteins are synthesized as sources of carbon, nitrogen and sulfur for the next generation of plants. Their composition changes according to nutritional conditions. Now, we report the precise molecular identification of seed proteins by proteomic analysis of wild-type *Arabidopsis thaliana*. Identities of 50 protein spots were determined in the protein extract of mature *Arabidopsis* seeds by two-dimensional (2-D) gel electrophoresis and subsequent mass spectrometric analysis. Of those protein spots, the spots up to 42 were identified to be derived from 12S globulins or 2S albumins. These results indicate that ca. 84% of protein species in *Arabidopsis* seeds are derived from few genes coding for 12S globulins and 2S albumins. Extensive mass spectrometric analysis on the 42 spots revealed that successive C-terminal degradation occurred on the 12S globulins. Two different N-termini were determined from 2S albumins. Feasibility of 12S globulins C-terminal processing was rationalized by molecular modeling of the three-dimensional structure of 12S globulins.

PSE-4 (Postdoctoral)

PHENOLIC ACIDS, GALLO-, AND ELLAGITANNINS FROM *PUNICA GRANATUM* L.

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Pomegranate (*Punica granatum* L.) has been shown to possess potent antioxidant activity, and is therefore believed to be able to prevent cancer, ageing, and other stress related diseases. Thus, pomegranate juice by-product (POMx) was investigated for active metabolites. Fractionation with EtOAc, *n*-BuOH and H_2O, and further purification of fractions on Amberlite XAD-16 and Sephadex LH-20 column chromatography yielded phenolic acids, and gallo- and ellagitannins. The bioactive metabolites were identified as gallic acid (**1**), glucogallin (**2**), hexahydroxydiphenic acid (**3**), ellagic acid (**4**), 2, 3–(*S*)-hexahydroxydiphenyl-□- D-glucopyranose (**5**), gallagyl dilactone (**6**), gallagic acid (**7**), punicalins (**8**), and punicalagins (**9**). The structures of the pure phenolic acids, and gallo- and ellagitannins were elucidated by NMR and MS techniques. These metabolites were evaluated for inhibition of ROS generation and cytotoxicity by cell based DCFH-DA and XTT assays, respectively. The metabolites inhibited ROS generation comparable to standards, and were not cytotoxic against HL-60 cells.

PSE-5

THREE NEW FLAVONOIDS FROM THE FERN *DRYOPTERIS VILLARII*

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Three new acylated flavonoid glycosides have been isolated from an ethanolic extract of aerial parts of the fern *Dryopteris villarii* (Bell.) Scinz & Thell by preparative paper chromatography followed by Sephadex LH-20 column chromatography. These products have been identified as kaempferol 3-*O*-(caffeoylrhamnoside) (**1**), apigenin 4′ -*O*-(caffeoylglucoside) (**2**) and apigenin 4′ -*O*-(feruloylglucoside) (**3**). Identifications were based on UV spectral analysis with the customary shift reagents, total acid hydrolysis (**1** gave kaempferol and L-rhamnose, whereas **2** and **3** gave apigenin and D-glucose) and alkaline hydrolysis (**1** gave caffeic acid and kaempferol 3-*O*-rhamnoside; **2** gave caffeic acid and apigenin 4′ -*O*-glucoside; **3** gave ferulic acid and apigenin 4′ -*O*-glucoside). The structures were confirmed by electrospray mass spectra (**1**: (m/z) 593 ([M-H.]$^-$), 431 (kaempferol glucoside) and 285 (kaempferol); **2**:(m/z) 593 ([M-H]$^-$), 431 (apigenin glucoside), 269 (apigenin) and 253 (glucosylated B-ring); **3**(m/z) :632([M+H+Na]$^+$), 455 (apigenin glucoside+Na) and 271 (apigenin)). *Dryopteris villarii* has a number of acylated flavonoid glycosides which are absent from *Dryopteris villarii* species with a single exception, the presence of kaempferol 3-*O*-(6′ ′ -succinylglucoside) in four *Dryopteris* species. Apigenin *O*-glycosides bearing an acylated sugar at the 4′ position are rare plant constituents.

PSE-6

APIGENIN 4□-O-(p-COUMAROYL GLUCOSIDE) FROM THE FERN DRYOPTERIS VILLARII

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A new flavonoid identified as apigenin 4′ -*O*-(*p*-coumaroylglucoside) (**1**) has been found in aerial parts of the fern *Dryopteris villarii* (Bell.) Scinz & Thell. This product has been isolated by preparative paper chromatography followed by Sephadex LH-20 column chromatography. Identification is based on UV spectral analysis with usual shift reagents, total acid hydrolysis (which gave apigenin and D-glucose), alkaline hydrolysis (which gave *p*-coumaric acid and apigenin 4′ -*O*-glucoside) and electrospray mass spectrum (positive mode) which showed a pseudomolecular ion at m/z 601 [M+Na]$^+$ and fragment ions at m/z 455 (apigenin glucoside +Na) and m/z 271 (apigenin). Kaempferol 3,7-di-*O*-rhamnoside (**2**) and kaempferol 3-*O*-(acetylglucoside)-7-*O*-rhamnoside (**3**) have also been found in aerial parts of this fern. Apigenin *O*-glycosides bearing an acylated sugar at the 4′ position are rare plant constituents. It has been reported [1] that flavonoid **2** has effects (hypertension and bradycardia) on rabbit cardiovascular system.

[1] Gohar,A.A., Elmazar,M.M.A. (1977) Phytotherapy Research 11, 564.

PSE-7

TAXANES OF THE FOLIAGE OF THE MEXICAN YEW, *TAXUS GLOBOSA*

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One of the four yews native to the western hemisphere and one of the most poorly known is the Mexican Yew, *Taxus globosa* Schlecht. It ranges from Central Nuevo León State in Northern México sporadically and as far South as Guatemala and El Salvador in Central America. Since its initial discovery, the species has been sporadically collected and has remained obscure in literature and rare in cultivation. These observations encouraged us to conduct this research to get information as complete as possible on the geographical distribution and present situation of the natural populations of *T. globosa*, allowing its conservation and domestication and also test the variation level within and between populations in the content of taxanes in the foliage. The purpose is to identify individuals remarkable in this trait for sustainable commercial use of this species. Taxol, deacetylbaccatine III and cephalomannine have been measured in the foliage of *Taxus globosa* trees growing in the shade of a forest canopy in seven populations along the Sierra Madre Oriental. Samples were analyzed by liquid chromatography and the data registered until now have shown that broad phenotypic variation exists both between and within populations in the taxanes content in the foliage of the tree species.

PPM-1 (Graduate Student)

FORMATION OF VOLATILE TERPENES IN ROOTS OF *ARABIDOPSIS THALIANA*

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Volatile terpenes are important tools in plant defense since they either directly ward off pathogens and herbivorous insects or attract natural enemies of insect pests (Pichersky and Gershenzon 2002). While many studies have concentrated on the function of terpenes in aerial parts of plants, the formation and role(s) of volatile terpenes in roots remain largely unknown.

Using *Arabidopsis thaliana* as a model, we have detected the emission of mono-(C_{10}), sesqui-(C_{15}) and diterpenes (C_{20}) in roots grown under axenic and hydroponic culture conditions. Treatment of *Arabidopsis* roots with jasmonic acid and the fungal root pathogen *Pythium irregulare* caused induced emission of the C_{11}-homoterpene DMNT (4,8-dimethylnona-1,3,7-triene). Since DMNT is commonly emitted from leaves in response to insect and pathogen attack and plays a role in indirect plant defense (Kappers et al., 2005), this volatile might also be important for the defense of plant roots against soil-borne pests.

We are currently in the process of identifying the terpene synthases (TPS) responsible for terpene volatile formation in *Arabidopsis* roots with a focus on the synthesis of DMNT and diterpenes. We further examine the roles of DMNT in plant defense against *Pythium irregulare* and the attraction of insect-parasitizing nematodes. A better understanding of the synthesis and function of root volatiles will aid in our general knowledge of interaction between plants and soil-borne organisms and may provide an alternative to current pest control practices.

Kappers, I.F. et al., (2005) *Science* **309**, 2070-2072.
Pichersky,E. and Gershenzon, J. (2002) *Curr.Opin.Plant Biol.* **5**, 237-243.

PPM-2

MEADOWFOAM-BASED BIOHERBICIDES

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The main focus of this study is to develop natural herbicides using meadowfoam (*Limnanthes alba*) products for organic farming. Meadowfoam is mainly grown in western Oregon for its seed oil. The economic value of meadowfoam lies in the seed oil which is a rich source of rare C_{20} and C_{22} fatty acids for the cosmetic and pharmaceutical industry. Meadowfoam seeds are also a rich source of the glucosinolate, glucolimnanthin (about 2-4% in the spent seeds). In the industrial-scale oil extraction process, myrosinase activity is intentionally destroyed in the seeds to prevent contamination of the seed oil with glucolimnanthin-derived aglycones. However, the aglycones have greater herbicidal activity than glucolimnanthin against grassy weeds, e.g., downy brome (*Bromus tectorum*), which is a prominent weed pest in eastern Oregon. We have developed a method for converting glucolimnanthin in enzyme-inactivated spent seeds (seedmeal) into the corresponding isothiocyanate by treating seedmeal with minute amounts of fresh, enzyme-active seeds. We have shown that this seedmeal product has enhanced levels of the isothiocyanate and that it has significantly greater herbicidal activity than unaltered, factory-grade seedmeal. We have also found evidence for the presence of a nitrile-forming enzyme in meadowfoam seeds that converts glucolimnanthin into the corresponding nitrile. Further experiments indicated that the nitrile has greater herbicidal activity than the glucolimnanthin-derived isothiocyanate in seed germination assays.