Phytochemical Society of North America

2006 PSNA Annual Meeting, July 8th-12th, 2006
Oxford, Mississippi

Program:

Saturday, July 8th

1:00 – 6:00 PM Registration (Yerby Conference Center Foyer)

4:00 – 6:00 PM PSNA Executive Committee Meeting (Yerby Conference Center)

6:00 – 9:00 PM Wine Mixer (Memory House)

Sunday, July 9th

Session I – Natural Product Synthesis and Biosynthesis, Part 1 (Chair: Daneel Ferreira)

8:00 – 8:10 AM Daneel Ferreira (University of Mississippi) – Opening Remarks

8:10 – 8:20 AM Alice Clark, UM Vice Chancellor for Research and Sponsored Programs – Welcome

8:20 – 9:00 AM Invited: Norman Lewis (Washington State University) – Plant phenolics in health-protection, disease treatment and plant growth/development: Biochemistry/structural biology of their formation

9:00 – 9:30 AM Neish: Steven Ralph , Joseph W. Hudgins, Sharon Jancsik, Lina L. Madilao and Jörg Bohlmann (University of British Columbia) – Functional genomics approaches to characterize the contribution of phenolic secondary metabolites to insect induced defenses in conifer trees

9:30 – 9:50 AM Contributed: Georg G. Gross **(**Universität Ulm) – Biosynthesis of hydrolysable tannins – Unraveled by enzyme studies

9:50 – 10:10 AM Contributed: Wei-Li Yang and Mark A. Bernards (The University of Western Ontario) – Aliphatic carbon flux analysis during wound-induced suberization

10:10 – 10:30 AM Break

10:30 – 11:10 AM Invited: Toni M. Kutchan (Leibniz Institute of Plant Biochemistry) – Plant natural products biosynthesis – Recent progress with alkaloids derived from dopamine

11:10 – 11:30 AM Contributed: Dylan Levac, Jun Murata and Vincenzo De Luca (Brock University) – Differential extraction of leaf epidermal enzymes by carborundum abrasion technique to purify, characterize and clone tabersonine 16-*O*-methyltransferase from *Catharanthus roseus*

11:30 – 11:50 AM Contributed: Daniel G. Vassão[1], David R. Gang[2], Eran Pichersky[3], Laurence B. Davin[1] and Norman G. Lewis[1] ([1]Washington State University, [2]University of Arizona, and [3]University of Michigan) Biosynthesis of phenolic flavor compounds in basil (*Ocimum basilicum*)

11:50 – 12:10 PM Contributed: Jack W. Blount[1], Luzia Modolo[1], Lahoucine Achnine[1, 2], and Richard A. Dixon[1] ([1]The Samuel Roberts Noble Foundation and [2]GenApps, Inc.) – A comparative study of *Medicago truncatula* glycosyltransferases involved in flavonoid and isoflavonoid biosynthesis

12:10 – 1:30 PM Lunch Provided at Yerby

Session II – Natural Product Isolation, Structure Elucidation, and Methods for Analysis (Chair: Charles Cantrell)

1:30 – 2:10 PM Invited: William F. Reynolds (University of Toronto) Overcoming Problems in Natural Product Elucidation by NMR

2:10 – 2:40 PM Neish: Xing-Cong Li, Yuanqing Ding, and Daneel Ferreira (University of Mississippi) – Absolute configuration and conformation of bioactive 3, 8´´-biflavonoids

2:40 – 3:00 PM Contributed: Jayaprakash, G. K.,[1] Mandadi, K.K.[1], Jadegoud, Y.[2], Nagana Gowda, G.A.[2] and Bhimanagouda S. Patil[1] ([1]Texas A & M University and [2] Centre of Biomedical Magnetic Resonance) – Unique anticancer limonoids from *Citrus aurantium*

3:00 – 3:20 PM Break

3:20 – 4:00 PM Invited: Rachel Mata, Sergio Martínez-Luis, Araceli Pérez-Vásquez and Rogelio Rodríguez. (Universidad Nacional Autónoma de México) – Natural phytotoxins with calmodulin inhibitor properties

4:00 – 4:20 PM Contributed: Amit Vikram, G. K. Jayaprakash, and Bhimanagouda S. Patil (Texas A & M University) – Simultaneous separation of health promoting bioactive citrus limonoids and their glucosides

4:20 – 4:40 PM Contributed: Lenong Li, Luzia Modolo, Richard A. Dixon, and Xiaoqiang Wang (Samuel Roberts Noble Foundation) – Crystal structure of a new uridine diphosphate glycosyltransferase from *Medicago truncatula*

4:40 – 5:00 PM Contributed: Zulfiqar Ali,[1] Shabana I. Khan,[1] Daneel Ferreira,[2] and Ikhlas A. Khan[1,2] (University of Mississippi) – Podocarpaside K, an arabinoside possessing a novel triterpenoid triterpenoid backbone from *Actaea podocarpa*

5:00 – 7:00 PM Business Meeting

Monday, July 10th

Session III – Natural Product Synthesis and Biosynthesis, Part 2 (Chair: Daneel Ferreira)

8:20 – 9:00 AM Invited: David G. I. Kingston (Virginia Polytechnic Institute and State University) – The Shape of things to come: Structural and synthetic studies of taxol and related compounds

9:00 – 9:20 AM Contributed: Jannie P. J. Marais and Daneel Ferreira (University of Mississippi) Synthesis of A-type proanthocyanidins

9:20 – 9:40 AM Contributed: Oliver R. A. Corea[1,2], Man-Ho Cho[1], Aldwin M. Anterola[1], Frances Anne Moog-Anterola[1], Susanne E. Kohalmi[2], Mark A. Bernards[2], Laurence B. Davin[1] and Norman G. Lewis[1] ([1]Washington State University and [2]University of Western Ontario) Phenylalanine biosynthesis in *Arabidopsis thaliana*: Identification and characterization of arogenate dehydratase(s)

9:40 – 10:10 AM Neish: Meimei Xu, Sladjana Prisic, Dana Morrone, Ross Wilderman, & Reuben J. Peters (Iowa State University) Gibberellins and beyond!

10:10 – 10:30 AM Break

10:30 – 11:10 AM Invited: Mitchell A. Avery, Blake Watkins, Kim Vines, Baogen Wu (University of Mississippi) - Progress towards the total synthesis of pseudolaric acid B, a novel anticandidal natural product from the bark of the yellow larch, *Pseudolarix kaempferi*

11:10 – 11:30 AM Contributed: Mary Magnotta, Jun Murata, Jianxin Chen and Vincenzo De Luca (Brock University) – Identification of a low vindoline accumulating

cultivar of *Catharanthus roseus* (L.) G. Don by monoterpenoid indole alkaloid and enzymatic profiling

11:30 – 11:50 AM Contributed: Syed G. A. Moinuddin, Michaël Jourdes, Laurence B. Davin and Norman G. Lewis (Washington State University) Towards defining interunit linkages sequences in lignin primary structure

11:50 – 12:10 PM Contributed: Kye-Won Kim, Syed G. A. Moinuddin, ChulHee Kang, Laurence B. Davin and Norman G. Lewis (Washington State University) Towards defining the molecular basis of differing enantiospecificities of pinoresinol-lariciresinol reductases in western red cedar using site-directed mutagenesis

12:10 – 1:30 PM Box Lunch Provided at Yerby

Session IV – Discovery and Development of Natural Products for Pest Management (Chair: Franck Dayan)

1:30 – 2:10 PM Invited: S. Textor, J-W. de Kraker, J. Tokuhisa, M. Reichelt, J. Gershenzon (Max Planck Institute for Chemical Ecology) – Engineering plant and chemical defense for managing insect pests: Lessons from *Arabidopsis*

2:10 – 2:30 PM Contributed: Raymond Thomas[1], Alice Fang[2], Carol A. Peterson[2], Terry R. Anderson[3] and Mark A. Bernards[1] ([1]The University of Western Ontario, [2]University of Waterloo and [3]Agriculture and Agri-Food Canada) – Suberin as a determinate of *Phytophthora* resistance in soybean

2:30 – 2:50 PM Contributed: C. Bertin, F. C. Schroeder, L. A. Weston (Cornell University) – Towards a new bioherbicide in turf settings?

2:50 – 3:20 PM Break

3:20 – 4:00 PM Invited: Stephen O. Duke, Franck E. Dayan, David E. Wedge, Agnes M. Rimando, Charles L. Cantrell, Kevin K. Schrader, Zhiqiang Pan, Scott R. Baerson, Nurhayat Tabanca, and Kumudini Meepagala (USDA, ARS) – Discovery and development of natural products for pest management

4:00 – 4:20 PM Contributed: Eric T. Johnson, Mark A. Berhow and Patrick F. Dowd (USDA-ARS) – *P1*-mediated transgenic secondary metabolite production in corn silks moderately enhances insect resistance

4:20 – 4:40 PM Contributed: Audrey Sauldubois[1], Charles L. Cantrell[2], Stephen O. Duke[2], Nidhi Singh[3], Chris McCurdy[3] and Franck E. Dayan[2] ([1]Université d'Angers, FRANCE, [2]USDA-ARS, Natural Products Utilization Research

Unit, and [3]University of Mississippi) – *p*-Hydroxyphenylpyruvate dioxygenase is a target site for β-triketone phytochemicals

6:00 – 9:00 PM Poster Session (National Center for Natural Products Research)

Tuesday, July 11th

Session V – Metabolic Engineering of Natural Products (Chair: Daniel Cook)

8:20 – 9:00 AM Invited: David Gang (University of Arizona) – Control of aromatic metabolism in sweet basil

9:00 – 9:20 AM Contributed: Jim Brandle, Alex Richman, Tania Humphrey, Ralph Chapman and Brian McGarvey (Agriculture and AgriFood Canda) – Identification, isolation and characterization of five steps in the synthesis of the sweet glycosides of *Stevia rebaudiana* using functional genomics

9:20 – 9:40 AM Contributed: Xiao-Hong Yu[1], Richard A. Dixon[2] and Chang-Jun Liu[1] ([1]Brookhaven National Laboratory and [2] the Noble Foundation) – Genome-wide characterization of acyl-CoA dependent acyltransferases responsible for (iso)flavonoid biosynthesis

9:40 – 10:10 AM Neish: Catherine Loncaric, Amanda Ward, and Kevin D. Walker (Michigan State University) – Evaluating the biogenesis and molecular pathways of bioactive plant products

10:10 – 10:30 AM Break

10:30 – 11:10 AM Invited: Sanja Roje (Washington State University) – Exploring and engineering plant one-carbon and folate metabolism

11:10 – 11:30 AM Contributed: Argelia Lorence[1], Bonnie J. Woffenden[2], Luis Nopo-Olazabal[1], Javier Martínez[1], Craig L. Nessler[2], and Fabricio Medina-Bolivar[1] ([1]Arkansas State University and [2]Virginia Tech) Enhanced production of specialized metabolites in tobacco over-expressing an AP2-type transcription factor

11:30 – 11:50 AM Contributed: David J. Schultz[1,2] and Oliver W. Starks[1] ([1]University of Louisville and [2]University of Louisville School of Medicine) – EST database approach to identify anacardic acid biosynthesis genes

11:50 – 1:30 PM Lunch Provided at Yerby

6:00 – 9:00 PM Banquet with Jones Sisters as entertainers (Oxford University Club)

Wednesday, July 12th

Session VI – Herbal Products and Nutraceuticals (Chair: Daneel Ferreira)

8:20 – 9:00 AM	Invited: Mahmoud ElSohly (University of Mississippi) – Marijuana project at the University of Mississippi
9:00 – 9:20 AM	Contributed: Keat T. Teoh, Devin R. Polichuk, Darwin W. Reed, Goska Nowak and Patrick S. Covello (Plant Biotechnology Institute) – Functional genomics and the biosynthesis of artemisinin
9:20 – 10:00 AM	Invited: Ikhlas Khan (University of Mississippi) – Marker compounds and beyond
10:00 – 10:30 AM	Break
10:30 – 11:10 AM	Invited: James D. McChesney (Tapestry Pharmaceuticals, Inc. and Chromadex Analytics, Inc.) – Natural products: Back to the future or into extinction?
11:10 – 11:40 AM	Neish: Fabricio Medina-Bolivar[1,2,4], John Hubstenberger[1], Jose Condori[1], Sean O'Keefe[3], Selester Bennett[4] and Maureen Dolan[1,4]. ([1]Arkansas Biosciences Institute, [2]Arkansas State University, [3]Virginia Tech and [4]Nature West Inc.) – Resveratrol production in hairy roots of peanut
11:40 – 12:00 AM	Contributed: 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) Bioactive metabolites of cranberry juice
12:20 – 1:40 PM	Box Lunch Provided at Yerby

Book of Abstracts

Oral Presentations

Norman G. Lewis. **Plant phenolics in health-protection, disease treatment and plant growth/development: Biochemistry/structural biology of their formation.**

The remarkable structural diversity of bioactive phenolics in various vascular plants has led to their growing usage in both health-protection and disease treatment. Examples include anti-oxidant properties of chlorogenic acid, as well as in widespread treatments of various viruses and cancers, such as with podophyllotoxin/nor-dihydroguaiaretic acid derivatives, and structural lignins. Other potent phenolics include those with antibacterial properties, e.g. eugenol, chavicol, etc. Using various plant systems, including *Arabidopsis*, all of the various enzyme isoforms involved in conversion of chorismic acid to the main phenylpropanoid pathway metabolites, the monolignols *p*-coumaryl, coniferyl and sinapyl alcohols, have been identified. Herein, we describe the interesting substrate versatilities of various downstream enzymes, such as *p*-coumaryl CoA shikimate quinic acid transferase and cinnamyl alcohol dehydrogenases, together with our current knowledge of their various catalytic mechanisms. Beyond the monolignol forming pathway, carbon can be specifically diverted into various natural products, such as to the bioactive dihydromonolignols, allyl- and propenylphenols, lignans and the structural lignins, depending on the species and tissues involved. For example, we briefly describe progress made in mechanistic studies of the recently discovered phenylpropenal double bond reductases, dirigent proteins, secoisolariciresinol dehydrogenase, etc. An additional emphasis is placed upon the analysis of various transgenic/mutant lines modified in lignin content and composition; these data indicate formation of regular primary lignin structures; the mode of lignin assembly, and how it is controlled, is thus also discussed.

Steven G. Ralph, Joseph W. Hudgins, Sharon Jancsik, Lina L. Madilao and Jörg Bohlmann.
Functional genomics approaches to characterize the contribution of phenolic secondary metabolites to insect induced defenses in conifer trees.

Tree species of the Pinaceae family have evolved a variety of anatomical and chemical, constitutive and inducible, direct and indirect defenses against insect pests and insect-vectored pathogens. In this regard, the tremendous structural diversity of terpenoid and phenolic secondary metabolites present in species of the Pinaceae family is of central importance to defense. In contrast to terpenoids, which have been well characterized at the anatomical, biochemical and molecular levels, the role of phenolic metabolites in constitutive and inducible conifer defense is more ambiguous. We have taken a functional genomics approach to characterize the role of phenolic metabolism in induced spruce (*Picea* spp.) defenses against the stem-boring insect, the white pine weevil (*Pissodes strobi*). Mining our large collection of Expressed Sequence Tags (ESTs) and high-accuracy full-length cDNAs (FLcDNAs) we have identified more than 2,700 ESTs and 200 FLcDNAs that collectively represent genes for nearly every known enzymatic step in the shikimate, stilbenoid/flavonoid, and lignin/lignan biosynthesis pathways in plants. Genome-wide transcript profiles of bark subjected to weevil feeding, generated using our spruce 21.8K cDNA microarray, has identified >100 induced transcripts associated with phenolic metabolism. Phylogenetic analysis of spruce FLcDNAs representing several gene families (e.g. P450 monooxygenases, dioxygenases, caffeic acid O-methyltransferases, dirigent proteins and chalcone/stilbene synthases), combined with refined real-time PCR expression profiling has identified specific genes for further biochemical characterization. In parallel, we have also examined the levels of phenolic metabolites in spruce bark attacked by weevils using Liquid Chromatography-Mass Spectrometry (LC-MS) for comparison with weevil-induced transcript changes. An integrated overview of this combined strategy of gene discovery, global transcript profiling, and phenolic metabolite profiling will be presented.

Georg G. Gross. **Biosynthesis of hydrolysable tannins – unraveled by enzyme studies.**

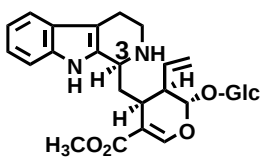
β-Glucogallin (1-*O*-galloyl-β-D-glucopyranose; βG) is the starter molecule in the biogenetic sequence to 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (PGG) that represents the central intermediate in the pathways to gallotannins and ellagitannins, the two subclasses of hydrolyzable plant tannins. Additionally, βG also serves as the principal galloyl donor in this route to PGG, as has been shown with enzymes isolated from oak leaves. Current sequencing experiments with the final enzyme of this series (converting tetragalloylglucose to PGG) indicate that it belongs to a group of serine carboxypeptidase-like acyltransferases that originated from proteases and acquired a new role in plant secondary metabolism. The same βG-dependent galloyltransferase mechanism was found to apply to the substitution reactions involved in the transformation of PGG to complex polygalloylglucoses (reaching deca- to undecagalloylated derivatives) in sumac (*Rhus typhina*). Five different enzymes have been isolated to date that produced a variety of hexa- and heptagalloylglucoses, plus unididentified higher analogues. Summarizing their substrate and product specificities, a scheme illustrating the primary steps in the routes to gallotannins was developed. Ellagitannins, in contrast, are the result of regio- and stereospecific oxidation reactions with PGG as the principal starter molecule. Two O_2-dependent phenol oxidases, isolated from fringe cups (*Tellima grandiflora*) leaves, catalyzed the sequence: PGG → tellimagrandin II (a monomeric ellagitannin) → cornusiin E (3, dimeric ellagitannin), thus providing first hard facts in support of decade-old theoretical considerations.

Wei-Li Yang and Mark A. Bernards. **Aliphatic carbon flux analysis during wound-induced suberization.**

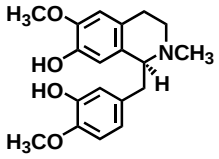
Suberin is a cell specific, wall-associated biopolymer formed during normal plant growth and development as well as in response to biotic and abiotic stresses. Suberized tissue is characterized by the deposition of both a poly(phenolic) domain (SPPD) in the cell wall and a poly(aliphatic) domain (SPAD) thought to be deposited between the cell wall and plasma membrane. The monomeric components that comprise the SPPD and SPAD have been well characterized by analyzing the compounds released after chemical degradation of suberized tissue. However, the biosynthesis of SPPD and SPAD components and their deposition during suberization is poorly understood. Using wound healing potato tubers as a model system, and targeted metabolite analysis, we have tracked the flux of carbon into the aliphatic monomers of the SPAD in a time course fashion, monitoring both soluble and insoluble components. From these analyses, we demonstrate that newly formed fatty acids undergo one of two main metabolic fates during wound-induced suberization: (1) desaturation followed by oxidation to form the 18:1 omega-hydroxy and dioic acids characteristic of potato suberin, and (2) elongation to very long chain fatty acids (C20 to C28), associated with reduction to 1-alkanols, decarboxylation to *n*-alkanes and minor amounts of hydroxylation. The partitioning of carbon between these two metabolic fates illustrates a high degree of metabolic regulation during wound healing, and provides insight into the organization of fatty acid metabolism.

Toni M. Kutchan. **Plant natural products biosynthesis – Recent progress with alkaloids derived from dopamine.**

The study of the biosynthesis of plant alkaloids at the enzyme and gene level has greatly advanced in recent years. We now have a number of genes available from the 3α(*S*)-strictosidine-derived monoterpenoid indole- and (*S*)-reticuline-derived tetrahydrobenzylisoquinoline alkaloid biosynthetic pathways. These gene sequences form the basis for identifying genes from other alkaloid pathways using molecular genetic techniques followed by functional expression and biochemical analysis. An update will be given on what we understand today about enzymes, genes and spatial organization in the tetrahydrobenzylisoquinoline alkaloid biosynthesis field and on how this, combined with knowledge of monoterpenoid indole alkaloid formation allows us to begin to investigate a new class of alkaloids with respect to biosynthetic genes and enzymes.



3α(*S*)-Strictosidine



(*S*)-Reticuline

Dylan Levac, Jun Murata and Vincenzo De Luca. **Differential extraction of leaf epidermal enzymes by carborundum abrasion technique to purify, characterize and clone tabersonine 16-O-methyltransferase from *Catharanthus roseus*.**

The monoterpene indole alkaloid (MIA) vindoline, an essential moiety of the antineoplastic agents vinblastine and vincristine, is formed from tabersonine through six sequential enzymatic steps in at least three different cell types. O-methylation of 16-hydroxytabersonine is catalyzed by an S-adenosylmethionine (SAM) dependent O-methyltransferase (OMT) that has yet to be cloned and characterized. Previous attempts to isolate 16-OH tabersonine OMT (16-OMT) from *Catharanthus* instead purified a flavonoid OMT from cell culture extracts that led to the biochemical characterization of a flavonoid-specific 4'OMT (Schröder, Phytochemistry, 2003, 62:127) instead of 16-OMT. Studies using laser capture microdissection (LCM) and carborundum abrasion (CA) techniques have shown that 16-OMT is expressed specifically in epidermal leaf cells of *Catharanthus* (Murata, Plant J, 2005, 44:581). The unique use of CA technique to enrich for epidermal proteins was combined with size exclusion, adenosine agarose affinity and anion exchange chromatography to purify of 16-OMT to homogeneity that was devoid of contaminating 4'-OMT activities. Peptide sequences obtained from 16-OMT were used to make appropriate primers to isolate a novel functionally active & unique 16-OMT clone with high homology to known OMTs from *Catharanthus*.

Daniel G. Vassão, David R. Gang, Eran Pichersky, Laurence B. Davin and Norman G. Lewis.
Biosynthesis of phenolic flavor compounds in basil (*Ocimum basilicum*)

Phenolic compounds, especially allylphenols, are among the principal chemical constituents of aromas from several herbs such as basil and anise, as well as other spices such as cloves and cinnamon. Plants accumulating these antimicrobial compounds have earned cultural and economic importance as foodstock protectants and spices, but the biosynthetic routes leading to their production *in planta* have remained unknown, despite decades of investigation. Most proposed biosynthetic pathways share steps with the phenylpropanoid pathway forming monolignols, but the final deoxygenation reaction had still to be elucidated. Here we report a biosynthetic pathway starting from phenylalanine as the C_6C_3 precursor that is transformed through the phenylpropanoid pathway into hydroxycinnamyl alcohols. The latter are reduced with intermediacy of an ester substrate that provides a more facile leaving group thus forming, in the Thai basil line chosen here, the side-chain deoxygenated product chavicol. This reaction proceeds in the presence of NADPH or NADH as hydride donors, and has shown some substrate versatility regarding the acid moiety in the ester substrate. Related work has led to the isolation of a reductase that catalyzes

the formation of eugenol. Further characterization of the enzyme(s) responsible for this reaction in thai basil is currently under way.

Jack W. Blount, Luzia Modolo, Lahoucine Achnine and Richard A. Dixon. **A comparative study of *Medicago truncatula* glycosyltransferases involved in flavonoidand isoflavonoid biosynthesis.**

Glycosyltransferases (GTs) transfer nucleotide-diphosphate-activated sugars to low molecular weight compounds, aiding in the stabilization, compartmentation and activation of a variety of secondary metabolites. *Medicago truncatula* is a model legume system which contains many secondary metabolites such as isoflavonoids, flavonoids, anthocyanins, and triterpene saponins which are glycosylated. An efficient HPLC method was developed to analyze the GT candidates against 32 potential substrates by dividing the substrates "equally" into four groups. The compounds in each group are readily separated by the HPLC method, and each compound within a particular group has a distinct UV spectrum. Using our extensive *M. truncatula* EST database, we have identified 63 full-length putative GTs which may be involved in the glycosylation of secondary metabolites. Soluble recombinant protein was purified for eighteen of the GTs using the Magne-His protein purification kit and assayed for activity using the four substrate mixtures. Eight of the eighteen GTs can glycosylate one or more of the flavonoid or isoflavonoid compounds. This poster will compare the glycosylation patterns of these eight GTs against a variety of individual flavonoid and isoflavonoid substrates.

William F. Reynolds. **Overcoming problems in natural product elucidation by NMR.**

The techniques for natural product structure elucidation by NMR are now well established and widely applied. However, there are potential problems, some not obvious, which may either lead to an incorrect assignment or the inability to unambiguously determine a structure. A number of these potential problems and ways of overcoming them will be discussed.

Xing-Cong Li, Yuanqing Ding and Daneel Ferreira. **Absolute configuration and conformation of bioactive 3,8"-biflavonoids.**

The 3,8″-biflavonoids represent a biosynthetically important group of natural products with significant biological activities. However, their absolute configuration and conformation remained undefined due to the complexity resulting from the restricted rotation along the C-3 and C-8″ interfalvanyl bond. In our search for antifungal agents from natural sources, several flavanone-(3→8″)-flavone type and flavanone-(3→8″)-dihydroflavonol type biflavonoids were isolated from plants. Careful analysis of their circular dichroism (CD) and NMR spectra permitted the assignment of their absolute configuration and preferred conformation in solution. Theoretical calculations of electronic CD spectra of these compounds using time-dependant density functional theory (TDDFT) with B3LYP/6-31G* basis set in Gaussian03 have further confirmed the assigned absolute configurations.

G. K. Jayaprakash, K. K. Mandadi, Y. Jadegoud, G. A. Nagana Gowda, and Bhimanagouda S. Patil. **Unique anticancer limonoids from *Citrus aurantium*.**

Evidences suggest that several citrus limonoids are known to possess anticancer and antiviral activities. Recently, the demand for minor and unique pure compounds in large quantities has been increased. To meet the growing need, a study has been conducted for the isolation, purification and identification of limonoids from the seeds of *Citrus aurantium.* Defatted seed powder was extracted with ethyl acetate and methanol for 8 h using Soxhlet type extractor successively. Both the extracts were concentrated under vacuum and loaded to adsorbent column chromatography separately. After repeated column chromatography, three compounds were obtained from EtOAc extract and one compound obtained from MeOH extract. The structures of the isolated compounds (1-4) were characterized by NMR and mass spectra as isolimonoic acid, limonexic acid, deacetylnomilin and deacetyl nomilinic acid glucoside. This project is based upon work supported by the USDA-CSREES under Agreement USDA-IFAFS # 2001-52102-02294 and USDA # 2005-34402-14401.

Rachel Mata, Sergio Martínez-Luis, Araceli Pérez-Vásquez and Rogelio Rodríguez. **Natural phytotoxins with calmodulin inhibitor properties.**

The Ca^{2+}/calmodulin (CaM) complex initiates a plethora of signaling cascades through the regulation of different enzymes and ion channels that culminate in alteration of the cellular functions. In higher plants this protein is a fundamental component of Ca^{2+} signal transduction pathway during germination and plant growth. Indeed, some phytotoxins seem to exert their potent phytotoxic activity due to their interaction with CaM. For example, ophiobolin A, a phytotoxin isolated from several species of the genus *Bipolaris*, is among the best known examples; this terpenoid interacts with CaM and inhibits its ability to activate CaM-sensitive enzymes such as PDE-1. During our systematic search for new herbicidal lead structures from natural sources, we have discovered several phytotoxins with CaM inhibitor properties from several fungi (*Guanomyces polythrix*, *Malbranchea aurantiaca*, *Phoma hebarum*) and plants (*Hofmeisteria schaffneri*, *Epidendrum rigidum* and *Flourensia cernua*). In this presentation an overview of our recent work on this topic will be discussed. Brief considerations on the natural sources as well as on the structure elucidation and CaM inhibitor properties of the phytotoxins will be presented.

Amit Vikram, G. K. Jayaprakash and Bhimanagouda S. Patil. **Simultaneous separation of health promoting bioactive citrus limonoids and their glucosides.**

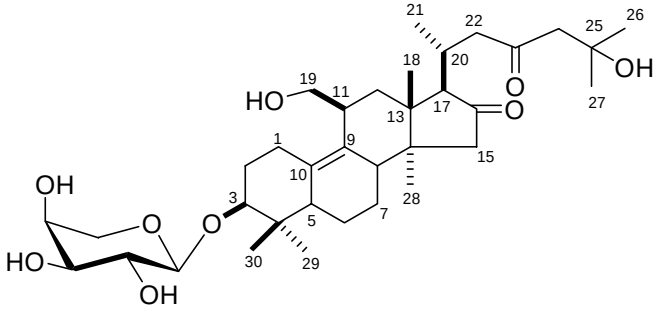
Limonoids are a large class of naturally occurring secondary plant metabolites and they are present in Rutaceae and Meliaceae families. Limonoids impart bitterness to citrus juices and not favored by citrus juice industry for long time. However, bitter limonoid aglycones turn to glucosides fruiting season progresses. The limonoid glucosides are tasteless and water soluble. Recent results showed that limonoids have many biological functions such as anticancer activity; antifeedant activity against insects and termites. It has been challenge to quantity both aglycones and glucosides simultaneously with higher precision. A simple, rapid and accurate high-performance liquid chromatography (HPLC) method was developed. The HPLC separation involves C-18 column with acetonitrile and aqueous phosphoric acid as the mobile phase and detection at 210 nm. The sensitivity of the method was precise with relative standard deviations for seven limonoids that ranged between 0.25 – 0.50 µg. The content of limonin and nomilin in citrus seeds were found comparable with the literature values. This method is expected to reduce cost of analysis and provide more precise results. This project is based upon work supported by the USDA-CSREES under Agreement USDA-IFAFS # 2001-52102-02294 and USDA # 2005-34402-14401 "Designing Foods for Health" through the Vegetable & Fruit Improvement Center.

Lenong Li, Luzia Modolo, Richard A. Dixon and Xiaoqiang Wang. **Crystal structure of a new uridine diphosphate glycosyltransferase from *Medicago truncatula*.**

The glycosyltransferases (GTs) catalyze the glycosylation reactions which are quantitatively the most important biochemical reactions on earth. In plants, many small molecular weight compounds can be modified by glycosylation catalyzed by family 1 uridine diphosphate glycosyltransferases (UGTs). These glycosylation processes can regulate the bioactivity of the compounds, their intracellular location and their metabolism.GT67A from *Medicago truncatula* has been recently identified as a glycosyltransferase with activity towards a number of phenylpropanoid-derived natural products. The crystal structure of GT67A was determined with molecular replacement using the program Phaser and the structure of UGT71G1 as a search model, and refined at 2.1 Å resolution. GT67A displays a similar GT-B fold observed previously for UGT71G1, and consists of two N- and C-terminal domains with similar Rossmann-type folds. The N-terminal domain has a seven-stranded parallel β sheet flanked by eight α-helixes. The C-terminal domain contains a six-stranded β sheet flanked by eight α-helixes. The two domains pack very tightly and form a deep cleft which is the binding site for substrates. Molecular docking using the program GOLD has been conducted to explore the binding of sugar donor and acceptor substrates. These results provide a structural basis for understanding substrate binding, specificity and possible catalytic mechanism for GTs in plant. The structural information will facilitate the rational design of GTs to improve the storage and stability of novel engineered bioactive compounds.

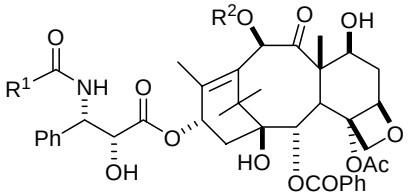
Zulfiqar Ali, Shabana I. Khan, Daneel Ferreira and Ikhlas A. Khan. **Podocarpaside K, an arabinoside possessing a novel triterpenoid triterpenoid backbone from *Actaea podocarpa*.**

Podocarpaside K, a novel arabinoside possessing a unique triterpene skeleton was isolated from *Actaea podocarpa*, a closely related species to black cohosh (dietary supplement used for menopausal disorders). Its structure was elucidated by extensive application of chemical and spectroscopic methods. Podocarpaside K belongs to a new class of triterpenoids, for which the name "ranunculane" is proposed. It showed anticomplement activity.

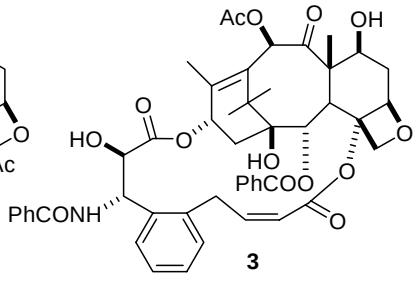


David G. I. Kingston. **The shape of things to come: Structural and synthetic studies of taxol and related compounds.**

Paclitaxel (Taxol™, **1**) and its semisynthetic analog docetaxel (**2**) are two of the most important anticancer agents developed over the last 30 years. Their primary mechanism of action is by interaction with the cellular protein tubulin, causing irreversible polymerization to microtubules. A detailed knowledge of this interaction is thus important in the design and development of analogs and also for the development of "non-taxane" tubulin polymerization agents. The lecture will review our work on discovering the tubulin-binding conformation of paclitaxel by a combination of REDOR NMR, fluorescence spectroscopy, and molecular modeling. This work has resulted in the design and synthesis of bridged paclitaxel analogs such as **3** that have activities superior to those of paclitaxel. The implications of this work for the future development of paclitaxel-like compounds will be discussed, and the synthesis of the simplified analog **4** will be described.



1 R^1 = Ph, R^2 = Ac
2 R^1 = Me_3CO, R^2 = H



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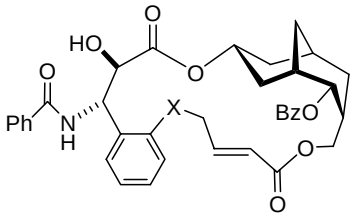

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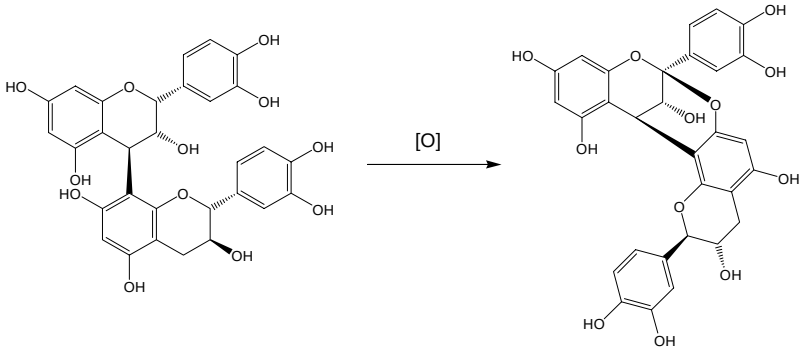
Jannie P. J. Marais and Daneel Ferreira. **Synthesis of A-type proanthocyanidins.**

There is a growing body of evidence to suggest that the beneficial effects of American cranberry (*Vaccinium macrocarpon*) juice against urinary tract infections is at least in part caused by the presence of A-type proanthocyanidins. Owing to the scant information that is available on the synthesis of this class of polyphenolic compounds, we are investigating methods to form A-type proanthocyanidins via oxidative conversion of their accompanying B-type analogs. Initial results aimed at synthesis of model-type precursors will be discussed.



Oliver R. A. Corea, Man-Ho Cho, Aldwin M. Anterola, Frances Anne Moog-Anterola, Susanne E. Kohalmi, Mark A. Bernards, Laurence B. Davin and Norman G. Lewis. **Phenylalanine biosynthesis in *Arabidopsis thaliana*: Identification and characterizataion of arogenate dehydratase(s).**

There has long been uncertainty as to how plants biosynthesize phenylalanine, i.e. in terms of whether arogenate, phenylpyruvate, or both serve as obligatory intermediates. That is, while both prephenate and arogenate have been reported in plants to undergo decarboxylative dehydration via the action of a dehydratase, neither the enzyme(s), nor the encoding gene(s) have been isolated and/or identified. In this study, a data mining approach was undertaken to attempt to identify the dehydratase(s) involved in Phe formation. Although differing quite markedly from their bacterial homologues, this approach suggested that there are 6 putative prephenate dehydratase (PDT) homologues in *Arabidopsis*, based on similarity of PDT-like and ACT-like domains to those from bacteria. Others have also suggested that these 6 genes may encode either PDT's or arogenate dehydratase (ADT's), but with no biochemical evidence for either function. Of the six putative ADT's or PDT's, 2 were cloned, with the corresponding recombinant proteins conveniently expressed in Nus-fusion form in *E. coli*. While the protein encoded by the first had a similar affinity (K_m) for both arogenate and prephenate, its overall catalytic efficacy was ~32 fold higher with arogenate (k_{cat}/K_m values of 7650 versus 240 M^{-1} s^{-1}, respectively); interestingly, the low level of PDT activity was inhibited by both Phe and Tyr. The second homologue more efficiently utilized arogenate, with its low PDT activity again being inhibited by Phe but not Tyr. Both proteins are thus provisionally classified as arogenate dehydratases, ADT1 and ADT2.

Meimei Xu, Sladjana Prisic, Dana Morrone, Ross Wilderman, & Reuben J. Peters. **Gibberellins and beyond!**

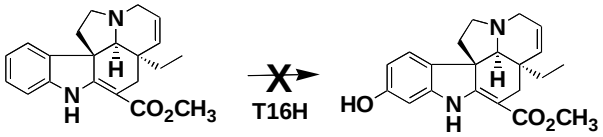
The central role of gibberellic acid (GA) phytohormones in plant growth and development, and the utility of GA metabolic mutants in the "Green Revolution", have stimulated intense interest in study of this biosynthetic pathway. A major focus of our research is enzymatic analysis of GA biosynthesis, which 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, highlighted by evidence for allosteric regulation of GA biosynthesis. 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. Rice produces more than ten such compounds, many of which serve as phytoalexins, via a complex biosynthetic network. Building on our biochemical studies of the *Arabidopsis* GA enzymes, we are utilizing a functional genomics approach to elucidate the enzymatic activity of the expanded families of 4 CPS, 12 KS, and 5 KO genes found in rice. Progress towards this goal will be presented, along with the associated findings of functional clustering of some of the consecutively acting diterpene synthases in the rice genome and phylogenetic evidence for the early derivation and widespread retention of labdane-related diterpenoid phytoalexin production in cereal crop plants.

Mitchell A. Avery, Blake Watkins, Kim Vines and Baogen Wu. **Progress towards the total synthesis of pseudolaric acid B, a novel anticandidal natural product from the bark of the yellow larch, *Pseudolarix kaempferi*.**

Pseudolaric Acid B, originally discovered as a chinese ethnobotanical antifungal agent for dermal application (Tu-Jin-Pe). It was later discovered to be a novel antipneumocystic and antifungal natural product from *Pseudolarix kaempferi* here at the National Center for Natural Products Research. Its potent activity against AIDS related *P. carini* and *C. albicans* opportunistic infections have promoted interest in its chemistry, structure-activity relationships, and mode of actions. Preliminary studies have lead to completed model studies and the total synthesis is well underway. Other interesting effects have shown an unexpected activation of peroxisome proliferators.

Mary Magnotta, Jun Murata, Jianxin Chen and Vincenzo De Luca. **Identification of a low vindoline accumulating cultivar of *Catharanthus roseus* (L.) G. Don by monoterpenoid indole alkaloid and enzymatic profiling.**



Tabersonine **16-Hydroxytabersonine**

The Madagascar periwinkle [*Catharanthus roseus* (L.) G Don] is a commercially important horticultural flower species and is the only source of the monoterpenoid indole alkaloids (MIAs), vinblastine and vincristine, key pharmaceutical compounds used to combat a number of different cancers. The present study uses high performance liquid chromatography for metabolic profiling of the MIAs extracted from seedlings and young leaves of 50 different flowering cultivars of *Catharanthus roseus* to show that, except for a single low vindoline cultivar (Vinca Mediterranean DP Orchid), they accumulate similar levels of MIAs. Further enzymatic studies with extracts from young leaves and from developing seedlings show that the low vindoline cultivar has a 10-fold lower Tabersonine-16-hydroxylase activity than those of *Catharanthus roseus* cv Little delicata. It is concluded that rapid metabolic and more selective enzymatic profiling of *Catharanthus* mutants could be useful for the identification of a range of MIA biosynthesis mutants.

Syed G.A. Moinuddin, Michaël Jourdes, Laurence B. Davin and Norman G. Lewis. **Towards defining interunit linkage sequences in lignin primary structure.**

Lignins, phenolic plant biopolymers present in vascular plant cell walls, are the most abundant substances after cellulose. Their structures and mode of biopolymer assembly from the three monolignols (*p*-coumaryl, coniferyl and sinapyl alcohols) have been the subject of unresolved debate for nearly 50 years. Two theories have emerged, namely non-random *vs.* random coupling and until recently, no experiments were designed to distinguish between them.

As a beginning to define lignin primary structure, the effects of modulating monomeric composition on the frequency of 8-*O*-4′, 8-1′, 8-8′ and 8-5′ inter-unit linkages in lignins from various transgenic plant

lines were studied. To do this, we synthesized various monomers/dimers that are cleaved/ released from lignins by thioacidolysis. For examples, various 8-1′ lignol dimers were synthesized using the Ivanoff reaction and then subjected to thioacidolysis/desulfurization. In an analogous manner, 8-8′ and 8-5′ dimeric compounds were enzymatically generated by dehydrogenation of *p*-coumaryl and coniferyl alcohols, whereas the 8-8′ dimer of S-S unit was chemically synthesized by $CuSO_4$-catalyzed oxidative dimerization of sinapyl alcohol. All dimers were individually subjected to thioacidolysis/desulfurization, and used as standards to determine interunit linkage frequencies in lignins. The data obtained for lignin analyses is interpreted as indicative of non-random assembly with defined cleavable inter-unit linkage sequence frequencies, which are invariant of monomer composition. These data illustrate the urgent necessity to develop methodologies further to sequence lignin primary structures.

Kye-Won Kim, Syed G.A. Moinuddin, ChulHee Kang, Laurence B. Davin and Norman G. Lewis.
Towards defining the molecular basis of differing enantispecificities of pinoresinol-lariciresinol reductases in western red cedar using site-directed mutagenesis.

Pinoresinol-lariciresinol reductases (PLRs) convert the 8-8′ linked lignan, pinoresinol, into secoisolariciresinol via lariciresinol; these metabolites confer protection against onset of various malignancies and their downstream product derivatives are also used widely in cancer treatment (e.g. podophyllotoxin). Two PLRs isolated from western red cedar, PLR_Tp1 and PLR_Tp2, share >71% identity, but each display quite different enantiospecifities, i.e. PLR_Tp1 preferentially utilizes the (–)-pinoresinol enantiomer, whereas PLR_Tp2 mainly processes the (+)-antipode. The X-ray crystallographic analysis of PLR_Tp1 and predicted PLR_Tp2 structures enabled us to model the cofactor (NADPH) and adjacent substrate binding sites. Based on these analyses, we have investigated the structure-function relationship of these enantiospecific conversions. We have identified four residues in the PLR catalytic domain that are important for enantiospecificity, mainly $Phe^{164}/Ser^{167}/Val^{268}/Leu^{272}$ for (–)-pinoresinol reductase (PLR_Tp1) and $Leu^{164}/Gly^{167}/Gly^{267}/Phe^{271}$ for (+)-pinoresinol reductase (PLR_Tp2). Substitution of the amino acid residues in PLR_Tp1 with the corresponding residues in PLR_Tp2 by site-directed mutagenesis resulted in an almost complete reversal of substrate enantiospecificity, i.e. to now mainly utilize the (+)-enantiomer over the corresponding (–)-antipode. Detailed kinetic analyses of wild type PLR_Tp1 and the corresponding mutant enzymes are described. These studies thus help enable us to identify the molecular basis for enantiospecificity in these conversions.

S. Textor, J-W. de Kraker, J. Tokuhisa, M. Reichelt, J. Gershenzon. **Engineering plant and chemical defense for managing insect pests: Lessons from *Arabidopsis*.**

Modern agricultural practices, such as the planting of monocultures of relatively undefended plants in the same location year after year inevitably lead to major insect problems. As the use of pesticides has given away to the direct manipulation of plant chemical defenses as the preferred method for reducing insect damage, it is worth remembering that wild plants have survived for millions of years under herbivore selection pressure. Hence the chemical defense patterns of wild plants may provide models worth emulating in agriculture. Here I use the distribution of glucosinolates in *Arabidopsis* thaliana to illustrate the typical profile of defense compounds in plants. Analysis of glucosinolate levels in different parts and developmental stages show that these compounds accumulate in highest concentrations in organs of most value, such as the youngest leaves (in the rosette stage) and the developing seeds. In addition, A. thaliana contains a complex mixture of glucosinolates rather than just one or two individual compounds due to the properties of its biosynthetic machinery. Mixtures are thought to increase resistance by promoting synergistic interactions among individual components and impeding the rate at which herbivores evolve resistance. By emulating these defense strategies in the development of new crop varieties, such as 2nd and 3rd generation Bt-transformants, plant breeders may reduce insect damage and increase the durability of resistance traits.

Raymond Thomas, Alice Fang, Carol A. Peterson, Terry R. Anderson and Mark A. Bernards. **Suberin as a determinate of *Phytophthora* resistance in soybean.**

Soybean (*Glycine max* L.) is the largest oilseed crop in the world. Each year, millions of dollars of potential yield revenues are lost due to root rot caused by the oomycete *Phytophthora sojae*. While gene-for-gene resistance exists for specific races of *P. sojae*, the main method for achieving field-level control is through breeding soybean varieties with quantitative trait loci (QTL)-mediated resistance. Since the root is the primary site of infection, we have examined suberization in soybean roots to determine whether this natural physico/chemical barrier correlates with tolerance to *P. sojae*. Chemical analysis of isolated soybean epidermis and endodermis tissues demonstrated increased amounts of suberin in (1) the epidermis along the root axis and (2) the endodermis as roots matured. Significantly higher amounts of suberin (P=0.05) were found in the tolerant cv 'Conrad' than in the susceptible cv 'OX760-6'. At the whole root level, a strong negative correlation between the amount of aliphatic suberin and field loss was observed for nine independent cultivars (r = -0.89) as well as 32 recombinant inbred lines (r = -0.87) derived from Conrad and OX760-6. Thus the amount of performed aliphatic suberin in the roots of Conrad is likely a primary determinate of QTL-mediated tolerance to *P. sojae*. As a contributor to *P.sojae* tolerance in some soybean cultivars, it may be possible to use root aliphatic suberin as a marker in selecting new varieties.

Bertin, F. C. Schroeder, L. A. Weston. **Towards a new bioherbicide in turf settings?**

TBA

Stephen O. Duke, Franck E. Dayan, David E. Wedge, Agnes M. Rimando, Charles L. Cantrell, Kevin K. Schrader, Zhiqiang Pan,, Scott R. Baerson, Nurhayat Tabanca, and Kumudini Meepagala. **Discovery and development of natural products for pest management.**

Our research group uses several strategies for discovery of natural products for pest management, including ethnobotanical, chemical ecology, chemical structure, and other biological activity clues. Furthermore, we try to test our compounds in a wide assortment of bioassays, including those for insecticides, herbicides, fungicides, algicides, and, in some cases, molluscicides. Our successes include discovery of tetranorclerodane insect repellants derived from the essential oil of *Callicarpa americana*. A systematic search for a selective blue-green algicide resulted in a highly effective 9,10-anthroquinone derivative. Two agricultural fungicides, sampangine and cyclopentenediones were discovered from compounds that were discarded as medicinal fungicides. The *p*-hydroxyphenylpyruvate dioxygenase-inhibiting triketones of manuka oil will be discussed as potential herbicides. Crops can be transgenically altered to synthesize new or greater amounts of natural compounds that could protect them from pests. The objective of one of our projects is to transgenically enhance production of the natural herbicide sorgoleone in sorghum. We have identified and cloned the putative genes for biosynthesis of this compound. We are beginning to develop a similar strategy for enhancing the production of protective compounds of rice. These and other aspects of our studies to discover natural compounds for pest management will be discussed.

Eric T. Johnson, Mark A. Berhow and Patrick F. Dowd. ***P1*-mediated transgenic secondary metabolite production in corn silks moderately enhances insect resistance.**

Secondary metabolites produced in corn silks can promote resistance to caterpillar pests. We evaluated Hi-II corn plants engineered to express the *P1* gene controlled by a putative silk specific promoter for secondary metabolite production and corn earworm resistance. Transgene expression did not enhance silk color, but 56% of newly emerged silks and 57% of mature silks displayed browning when cut, which indicates the presence of *P1*-produced secondary metabolites that are substrates of silk peroxidases. Levels of maysin, a secondary metabolite with insect toxicity, were present on average at 0.44% fresh weight (FW) in newly emerged silks and averaged 0.12% FW in mature silks compared to 0.008% FW and 0.002% FW in non-browning newly emerged and mature silks, respectively. Some

transgenic kernels browned spontaneously, suggesting the promoter may not be silk specific or that genetic rearrangements occurred that conferred *P1* expression in these kernels. The average mortality of corn earworm larvae fed newly emerged browning silks (61%) was not significantly different from the mortality of those fed newly emerged non-browning silks (53%). Mean survivor weights of corn earworm larvae fed mature browning silks (0.34 mg) were significantly lower than weights of those fed mature non-browning silks (0.39 mg). This information suggests *P1* expression in silks can moderately enhance insect resistance in mature silks, and that maysin production promoted by *P1* expression may be a contributing factor.

Audrey Sauldubois, Charles L. Cantrell, Stephen O. Duke, Nidhi Singh, Christopher McCurdy and Franck E. Dayan. **Herbicidal activity of manuka oil is associated with inhibition of p-hydroxyphenylpyruvate dioxygenase.**

p-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme in tyrosine catabolism and is the molecular target site of β-triketone pharmacophores used to treat hypertyrosinemia in humans. In plants, HPPD is involved in the biosynthesis of prenyl quinones and tocopherol, and is the target site of β-triketone herbicides. The β-triketone-rich essential oil of manuka (*Leptospermum scoparium*), and its components leptospermone, grandiflorone and flavesone were tested for their activity in whole-plant bioassays and for their potency against HPPD. The phenotype of developing plants exposed to manuka oil or its purified β-triketone components was similar to that of plants exposed to a synthetic HPPD inhibitor. Chlorophyll and carotenoid levels decreased in a dose-dependent manner upon inhibition of HPPD. Unlike their synthetic counterpart, steady-state O_2 consumption experiments revealed that the natural triketones behaved as competitive reversible inhibitors of HPPD, with the relative inhibitory activities of grandiflorone > leptospermone > flavesone. Structure-activity relationships indicate that the size and lipophilicity of the side chain affected the potency of the compounds. Computational analysis of the catalytic domain of HPPD indicates that a lipophilic domain proximate from the Fe^{2+} favors the binding of ligands with lipophilic moieties.

David Gang. **Control of aromatic metabolism in sweet basil.**

Sweet basil (*Ocimum basilicum*) plants produce many compounds in their peltate glandular trichomes that are important not only for plant defense, physiology, and interaction with the environment but that are also highly valued as flavoring/fragrance additives and as medicinal compounds. For example, eugenol and related volatile phenylpropenes are important components of many economically important spices, and they act in defense against animals and microorganisms and as floral attractants of pollinators. Similar properties have been observed for methylcinnamate and for rosmarinic acid, which has been shown to be a potent antioxidant and potential anti-inflammatory agent. We have identified the genes encoding enzymes involved in production of many of these compounds. Most recently, we have identified cinnamate carboxyl methyltransferase (CACMT), which produces methylcinnamate, and eugenol synthase (EGS), which uses esters of coniferyl alcohol to form eugenol and of *p*-coumaryl alcohol to form chavicol. In addition, we have identified the acyltransferase responsible for formation of the substrates for EGS. The relationships of these enzymes to other members of their respective protein families, their substrate preferences, and a discussion of their catalytic mechanisms will be presented.

Jim Brandle, Alex Richman, Tania Humphrey, Ralph Chapman and Brian McGarvey. **Identification, isolation and characterization of five steps in the synthesis of the sweet glycosides of *Stevia rebaudiana* using functional genomics.**

Stevia rebaudiana leaves accumulate a mixture of at least eight different steviol glycosides. The aglycone steviol is formed from a branch of the gibberellic acid biosynthetic pathway by C-13 hydroxylation of ent-kaurenoic acid. The pattern of glucosylation of steviol heavily influences the taste perception of these intensely sweet compounds. The majority of the glycosides are formed by four glucosylation reactions that start with steviol and end with rebaudioside A. We used our collection of ESTs, enzyme motif specific electronic probes and key word searches to identify candidate P450 and

glucosyltransferase genes resident in our collection. We isolated full length cDNAs for both candidate glucosyltransferases and P450s, cloned them into expression vectors in *E. coli* for the UGTs and yeast for the the P450s, then produced recombinant enzymes. Assays with authentic substrates were then performed and HPLC and LC-ES/MS were used to identify reaction products. We have now isolated and characterized kaurene oxidase, kaurenoic acid 13-hydroxylase and three of the four glucosyltransferase enzymes involved in steviol glycoside synthesis. In addition the subcellular location of both KO and three of the UGTs involved in steviol glycoside biosynthesis was investigated by expression of GFP fusions and cell fractionation, which revealed KO to be associated with the endoplasmic reticulum and the UGTs in the cytoplasm. It has also been shown by expressing the *Stevia* UGTs in *Arabidopsis* that the pathway can be partially reconstituted by recruitment of a native *Arabidopsis* glucosyltransferase.

Xiao-Hong Yu, Richard A. Dixon and Chang-Jun Liu. **Genome-wide characterization of acyl-co-A dependent acyltransferases responsible for (iso)flavonoid biosynthesis.**

Acyl-CoA dependent acyltransferases catalyze the transfer of aliphatic and /or aromatic acyl moiety from CoA thioesters to the nucleophile (OH- or NH-) of acceptor molecules. In plants, enzymatic *O*-acylation widely occurs in the formation and modification of a variety of plant secondary metabolites. Flavonoids and isoflavonoids are a large family of polyphenolics that are involved in flower pigmentation, UV radiation protection, defense resistance to fungal pathogen infection, and signaling for *Rhizobium* symbiosis and VAM-colonization. Substitution of acyl group on flavonoid and isoflavonoid compounds often alters the compounds' stability and solubility, and promotes a high-level accumulation in central vacuole of plant cells. Based upon the conserved sequence signatures of acyltransferase superfamily (BAHD), a total of about 39 tentative genes have been identified from *Medicago truncatula* EST databases. To functionally characterize this large number of putative acyltransferases and identify the enzymes responsible for isoflavonoid biosynthesis, we have developed a high throughput analytic procedure by combining the efficient Gateway cloning for vector construction, magnetic-HIS system for rapid protein extraction and purification, 96 well-micro titer plate-formatted *in vitro* assay, and on-line product detection and identification by HPLC-ESI-MS. Several (iso)flavonoid biosynthetic malonyltransferases and acetyltransferases have been characterized. The *in vivo* biological functions are being explored with gene over-expression and the results will be discussed. Characterization of (iso)flavonoid biosynthetic acyltransferases implicates the potential applications in genetic engineering of plant disease resistance or high–level production of health promoting nutraceuticals.

Catherine Loncaric, Amanda Ward and Kevin D. Walker. **Evaluating the biogenesis and molecular pathways of bioactive plant products.**

Taxol, from *Taxus* plants, plays a key role in treating pathological conditions, including cancer, heart disease and Alzheimer's. As applications for this important pharmaceutical continue to increase, it becomes important to maintain a balance between production demands and preservation of the *Taxus* flora. This concern can likely be addressed by up-regulating the metabolic pathway in planta through bioengineering techniques to make more of the desired compound, or alternatively, the segments of the pathway potentially can be moved to a suitable-production host, like *E. coli* to manufacture portions of the metabolite or second-generation analogs. To achieve either of these goals, the genes of the pathway must be isolated and the corresponding enzymes characterized. Technological advances in molecular genetics (e.g., EST and differential display methods), metabolite analysis, and enzyme assay development have streamlined the process of dissecting the biosynthetic pathways of Taxol, and its related metabolites. Nineteen genes are estimated to lie on the Taxol pathway; the isolation and characterization of six of the genes (five acyltransferases and an aminomutase) are briefly discussed, along with the application of these gene products in the production of unnatural compounds.

Sanja Roje. **Exploring and engineering plant one-carbon and folate metabolism.**

Nutritional folate deficiency has been linked to cardiovascular disease, birth defects, and cancer world-wide. Because of the known health risks associated with folate deficiency, all enriched cereal-grain foods in the U.S. are fortified with folic acid, a synthetic compound that is converted to natural folates in the body. This fortification succeeded in reducing the rate of birth defects; and in lowering the average blood levels of the amino acid homocysteine, an independent risk factor for cardiovascular disease. This fortification, however, is impractical in many developing countries. Moreover, concerns exist about the adverse effects of folic acid fortification on some population segments. These adverse effects include masking the symptoms of vitamin B_{12} deficiency, and accelerated growth of pre-existing tumors. Because of these adverse effects, many industrial countries do not practice folic acid fortification despite the associated health risks. The health problems associated with folate deficiency and folic acid fortification could be minimized or eliminated by enriching human diets with natural folates. Plants, able to synthesize folates *de novo*, are the main source of these vitamins in the human diet. Engineering of folate-enriched plants is thus an appealing choice to improve folate intake in humans. Hence, the long-term goal of my laboratory is to enable fortification of the human diet with natural folates by engineering enriched crops. Toward that goal, we are investigating the metabolism of tetrahydrofolate-bound one-carbon units, and developing engineering strategies to create folate-enriched plants. Natural folates comprise more than fifty derivatives of the cofactor tetrahydrofolate. My laboratory aims to enrich plants in a subset of folate derivatives by genetically manipulating the enzymes that act on the tetrahydrofolate-bound one-carbon group.

Argelia Lorence, Bonnie J. Woffenden, Luis Nopo-Olazabal, Javier Martínez, Craig L. Nessler and Fabricio Medina-Bolivar. **Enhanced production of specialized metabolites in tobacco over-expressing an AP2-type transcription factor.**

Plant specialized metabolites have been used as phytomedicines for hundreds of years. It is well known that multiple biosynthetic routes leading to the formation of these compounds are inducible and hypothesized that key regulatory proteins act as major orchestrators of the specialized metabolism. The transcription factor ORCA3 identified from *Catharanthus roseus* is a master regulator of genes involved in both primary and secondary metabolism. ORCA3 enhances the expression of genes downstream of the stress- and wound-inducible plant hormone methyl jasmonate (MeJA). We cloned an ORCA3 homolog from *Nicotiana tabacum* (named ORNA), constructed cassettes for its constitutive and inducible expression, and generated tobacco transgenic plants. Confirmation of the transformation event has been carried out via PCR, and ORNA over-expressers have been identified via semi-quantitative RT-PCR. Enhanced production of an inducible metabolite has been detected via TLC in a chloroform extract of the media where tobacco ORNA over-expressing plants were grown hydroponically. Identity of the inducible metabolite(s) is being conducted. Our data suggest that over-expression of ORNA led to activation of a specific inducible metabolic pathway in tobacco.

David J. Schultz and Oliver W. Starks. **EST database approach to identify anacardic acid biosynthesis genes.**

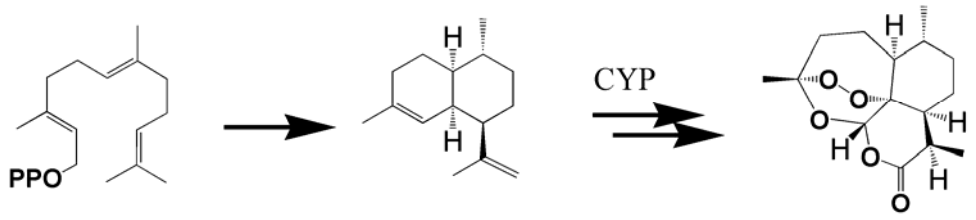
Anacardic acid (2-hydroxy-6-alkylbenzoic acid) is a group of related molecules that differ with respect to alkyl chain length, double bond placement and/or the number of double bonds in the alkyl group. Anacardic acid (AnAc) is known to occur in a number of plant families (most commonly found in *Anacardiaceae*) and has been reported to have a wide range of biological activities (inhibition of tumor cell growth, bacterial growth and fungal spore germination). In most plants, AnAc is one component in a complex mixture of phytochemicals including related phenolic lipids (e.g. alkyl-resorcinols). However, *Pelargonium* × *hortorum* (geranium) produces and secretes relatively pure AnAc exudates from glandular trichomes and thus may provide a less complex system for studying the bioactivity and biosynthesis of AnAc. We have previously demonstrated purified AnAc can disrupt development of Colorado potato beetle larvae when given as part of a diet. Thus, AnAc may have potential to impart pest resistance through bioengineered production in crop plants. Since biosynthesis of AnAc likely involves multiple

metabolic pathways (*e.g*. fatty acid synthesis and polyketide synthesis), we have initiated an EST database approach to identify and characterize all sequences involved in production of this phytochemical. We are currently building a geranium trichome EST database and are screening for sequences likely to be involved in AnAc biosynthesis. Since AnAc is only produced in trichomes of geranium we will use expression patterns to prioritize identified sequence for further biochemical analysis.

Mahmoud A. ElSohly. **Marijuana project at the University of Mississippi.**

Marijuana, the crude drug derived from the cannabis plant (*Cannabis sativa*) is the most widely abused drug in the world. Studies with marijuana go back for centuries and the drug was indicated for the treatment of a variety of ailments. However, it was only in 1964 that the chemical structure of the active ingredient (Δ^9-THC) was finally elucidated. This stimulated much research with the drug and its constituents, mainly the cannabinoids. The University of Mississippi has been, since 1968, the source of cannabis raw materials through contract with the National Institute on Drug Abuse (NIDA). This presentation will elaborate on the activities involved in meeting the requirements of the contract as well as related product development activities.

Keat T. Teoh, Devin R. Polichuk, Darwin W. Reed, Goska Nowak and Patrick S. Covello. **Functional genomics and the biosynthesis of artemisinin.**



farnesyl diphosphate amorpha-4,11-diene artemisinin

Artemisinin, a sesquiterpene lactone endoperoxide derived from *Artemisia annua*, forms the basis of the most important treatments of malaria in use today. In an effort to elucidate the biosynthesis of artemisinin, an expressed sequence tag approach to identifying the relevant biosynthetic genes was undertaken using isolated glandular trichomes as a source of mRNA. Progress in the elucidation of genes involved in artemisinin biosynthesis will be discussed, including the isolation of a cDNA encoding a cytochrome P450 involved in amorpha-4,11-diene oxidation.

Ikhlas A. Khan. **Marker compounds and beyond.**

The surging interest in botanical medicines in the United States has resulted in new challenges, since the approaches utilized in evaluating and approving prescription drugs are not directly or readily applicable to these complex mixtures. The scientific issues range from careful evaluation of traditional uses and proper identification of the plant, to ensuring purity and potency of the final dosage forms and the safety and efficacy of the dosing regimen. Establishing the pharmacological mechanisms of the majority of herbal remedies is another challenge that requires an active multidisciplinary scientific collaboration. Some approaches to the solution of quality problems with botanical medicines are outlined, with illustrative examples. First, there is a real need for the development of newer analytical approaches (HPLC, GC, and CE) with modern detection modalities. That will be pivotal tools for the rigorous scientific evaluation of botanical products, whether applied to active constituents or to surrogate markers. Second, the accessibility of marker compounds or reference standards is critical. In absence of these compounds, the potency, safety, and efficacy of herbs cannot be rigorously assessed, whether by biological or by chemical means. Third, chemical methods can be combined with pharmacological characterizations to ascertain the active principle, or to determine additive, synergistic, or antagonistic interactions of constituents. Fourth, approaches to evaluating authenticity of the source plant material are

currently being explored by combining the aforementioned analytical tools with genetic characterization. This can be applied to many problems, including the analysis of geographically diverse species in order to characterize the variability and guide selection of raw materials. Last, we have applied analytical methods to identify and evaluate pesticide and heavy metal content in a number of products.

James D. McChesney. **Natural products: Back to the future or into extinction?**

Natural product substances have historically served as the most significant source of new leads for pharmaceutical development. However, with the advent of robotics, bioinformatics, high throughput screening (HTS), molecular biology-biotechnology, combinatorial chemistry, in silico (molecular modeling) and other methodologies, the pharmaceutical industry has moved away from natural products as a source for leads and prospective drug candidates. Can, or will, natural products ever recapture the preeminent position once held as a foundation for drug discovery and development? I will discuss the challenges associated with development of natural products as pharmaceuticals with illustrative examples from the Taxol story and identify various perceptions that must be addressed to return natural products to the forefront of discovery and development of pharmaceuticals.

Fabricio Medina-Bolivar, John Hubstenberger, Jose Condori, Sean O'Keefe, Selester Bennett and Maureen Dolan. **Resveratrol production in hairy roots of peanut.**

Resveratrol is a popular, natural antioxidant molecule associated with cardiovascular and anticancer health benefits. While resveratrol can be recovered as an extract from a variety of plants, these products are not suitable for many applications in the food/pharmaceutical sectors due to impurities, low concentrations and associated color. To explore the utility of hairy roots in delivering a high quality resveratrol product to these industries, hairy root cultures of peanut (*Arachis hypogaea*) were established via *Agrobacterium rhizogenes*-mediated transformation. Several elicitors were tested for induction of resveratrol. Different elicitors induced biosynthesis of a distinct profile of stilbenoids, including resveratrol and putative resveratrol derivatives. Resveratrol was secreted into the medium of the hairy root cultures and detected by thin layer chromatography and fluorescence analyses in reference to authentic standards. Our results indicate that peanut hairy root cultures may provide a bioprocessing system for high quality production of resveratrol and resveratrol derivatives.

Christina M. Coleman, Daneel Ferreira, Amy B. Howell, Jess D. Reed, Christian G. Krueger and Jannie P.J. Marais. **Bioactive metabolites of cranberry juice.**

Compounds found in cranberry juice (*Vaccinium macrocarpon*) have been shown to prevent the adherence of P-fimbriated uropathogenic bacteria to uroepithelial cells *in vitro* and 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. These compounds have been identified 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, their urinary metabolites are currently unidentified. Urine was therefore collected over a 24 hr period from an adult female sow fed 800 grams of lyophilized cranberry juice powder per day for seven days prior to collection. Bioassay guided fractionation of the urine sample was then used to isolate the bioactive components using Sephadex LH-20 and other chromatography techniques. While the exact bioactive metabolites have not yet been conclusively identified, they are believed to be either glucuronated or sulfated derivatives of proanthocyanidin trimers or oligomers. After further isolation and purification, the metabolites will be identified and structurally characterized using NMR, MS and other spectroscopic techniques.

Poster Abstracts

01. *Berhow, Mark A., Eric T. Johnson, Patrick F. Dowd and Sandra M. Duval.* **Extraction and characterization of anthocyanins from pigmented flowers and black seed hulls.**

New methodology has been developed for the preparative scale extraction and purification of anthocyanins from "black" seed coats and hulls. A combination of physical fractionation, extraction, flash chromatography and preparative chromatography has resulted in the isolation of gram quantities of purified anthocyanins from petunia and gentium flowers, and black soybeans, black beans and cuphea seed hulls. The anthocyanin structures were determined by LC-MS. The methodology developed can be used to quantitate the levels of the anthocyanins in various cultivars and growing conditions. The quantities being isolated allow for the evaluation of their insecticidal, fungicidal and herbicidal activities in a variety of bioassay systems.

02. *Case, Bethany, David Wedge, Charles Cantrell, E. Edward Mena and Stephen O. Duke.* **Ascomycetes and basidiomycetes: Sources of novel agricultural fungicide agents.**

The Natural Products Utilization Research Unit of ARS began collaboration with LifePharms Inc. in 2004 to evaluate its extensive collection of ascomycete and basidiomycete fruiting body extracts for natural antifungal compounds; potential leads for new agrochemicals. Extracts were evaluated for their antifungal activity against *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*. Approximately 6,000 of the projected 13-16,000 samples have been evaluated. Of the extracts tested, 38 have been designated primary lead candidates with activity against all the fungal isolates, 335 secondary lead candidates with activity over at least one isolate, and 216 extracts of interest with varied and unique active zones over some or all isolates. Research is currently underway to isolate fungicidal compounds from lead candidates by a bioassay-guided process.

03. *Chang, Zhenzhan, Xianzhi He, Hui Shao, Richard A. Dixon and Xiaoqiang Wang.* **Structural studies of two enzymes involved in isoflavonoid phytoalexin biosynthesis.**

Isoflavonoids are legume natural products produced via the isoflavonoid branch of the phenylpropanoid metabolic pathway. They may play important roles in plant defense as phytoalexins, and also have significant benefits for human and animals. Isoflavone synthase (IFS) and isoflavone reductase (IFR) are two key enzymes involved in the biosynthesis of medicarpin, the major isoflavonoid phytoalexin in alfalfa responding to fungal pathogen attack. IFS, a P450 monooxygenase, catalyses the entry-point reaction into isoflavonoid biosynthesis. IFR is a NADPH-dependent reductase and converts 2'-hydroxyformononetin to (3R)-vestitone. We recently determined the crystal structure of alfalfa IFR at 1.6 Å resolution. To further explore the roles of the potential key residues defined from the molecular modeling and docking studies, structure-directed point mutagenesis and kinetic studies were conducted targeting residues in direct contact with the co-factor NADPH and the substrate 2'-hydroxyformononetin. These studies confirmed the role of Lys 144 as a general base, the role of Gly 14 of the fingerprint region in recognition of NADPH, and the roles of some other key residues for binding with co-factor and substrate. We are also investigating IFS, a key player in isoflavonoid biosynthesis. IFS was cloned into an *E. coli* vector for protein expression and purification towards crystallographic study. Some preliminary results will be presented.

04. *Demirci, Betul, Kemal Husnu Can Baser, Nurhayat Tabanca and David E. Wedge.* **Characterization of volatile constituents of *Haplopappus greeneei* and studies on the antifungal activity against phytopathogens.**

Essential oil of *Haplopappus greenei* A. Gray was obtained by hydrodistillation of aerial parts, which were subsequently analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Major components were identified as carvacrol (8.7%), β-pinene (7.6%), *trans*-pinocarveol (6.2%), and caryophyllene oxide (5.8%), respectively. In total, 104 components representing 84.9% of the investigated essential oil were characterized. Furthermore, the essential oil was evaluated for

antimalarial, antimicrobial, and antifungal activities. However, only antifungal activity was observed against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using the direct overlay bioautography assay. Major essential oil components were also evaluated for antifungal activity and the carvacrol standard demonstrated non-selective activity against the three *Colletotrichum* species and the other compounds were inactive.

05. *Guerra, Damian, Fiona Cochrane, Diana L. Bedgar, Laurence B. Davin and Norman G. Lewis.* **Characterization of a putative bifunctional CCR/CAD in *Arabidopsis thaliana.***

In silico analysis of the *Arabidopsis* genome sequence databases indicates that there are some 11 putatively annotated cinnamoyl CoA reductases (CCR) homologues, AtCCR1 – AtCCR11. With the goal of clarifying the physiological/biochemical role of each, particularly as regard monolignol biosynthesis, all 11 CCR cDNAs were obtained from *A. thaliana*. AtCCR1, 2 and 8 were heterologously expressed in *E. coli*, with the corresponding recombinant proteins purified to apparent homogeneity. Detailed biochemical characterization of each was carried out using cinnamoyl, *p*-coumaroyl, caffeoyl, feruloyl, 5-hydroxyferuloyl and sinapoyl CoAs as substrates. Of the three, both AtCCR1 and 2 had CCR properties, whereas AtCCR8 displayed both CCR and cinnamyl alcohol dehydrogenase (CAD) activities. This contribution summarizes our current knowledge of the biochemical (kinetic parameters) data for each isoform investigated thus far.

06. *Hale, Amber L., Stephen O. Duke, Ikhlas A. Khan, Charles L. Cantrell.* **Phytochemical investigations of the convolvulaceae.**

Convovulus arvensis and several species of the genus *Ipomoea* are reported to significantly inhibit the germination of a variety of monocots and dicots. Saponins, flavonoids, alkaloids, and lipids have been identified from extracts of *C. arvensis*. In an effort to find new compounds that could potentially be used as herbicides, crude extracts of aerial parts and seeds from members of the Convolvulaceae family were evaluated for phytotoxicity against *Lactuca sativa* (lettuce) and *Agrostis stolonifera* (bentgrass) seeds. Species screened included members from the following genera: *Convolvulus*, *Ipomoea*, *Maripa*, *Evolvulus*, and *Aniseia*. Ethanol extracts from species of each genera demonstrated a low level of activity at 1 mg/mL, while extracts from the most active species gave almost complete inhibition of germination at 1 mg/mL. In an effort to identify the bioactive constituents using a bioassay-guided approach, the most active extract was extracted sequentially with hexanes, methylene chloride, ethanol, and water. The active EtOH fraction was then further partitioned by liquid-liquid extraction. The EtOAc layer proved to be active, and bioassay-guided fractionation led to the identification of two active column fractions that will be further purified using LH-20 and preparative HPLC. Structure elucidation will be performed using mass spectrometry and one- and two-dimensional NMR techniques.

07. *Jackson, L., F. Chen, J. Nakashima, G. Shadle, M.S.S Reddy, and R. Dixon.* **Studying monolignol biosynthesis in alfalfa by the down-regulation of *cinnamoyl CoA reductase*.**

Lignin provides strength to plant stems and hydrophobicity to facilitate the transport of water through vascular tissues. Lignin is composed of primary building blocks called monolignols. The primary monolignols are guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) units. Due to the resistance of lignin to chemical breakdown, and its negative impacts on forage digestibility and paper pulping, the genetic manipulation of lignin quantity and/or quality is of great interest. Our group is currently studying multi-site modulation of lignin biosynthesis in the forage legume alfalfa (*Medicago sativa* L.), with a view to both better understanding the monolignol pathway in this important forage species, and determining the best targets for lignin modification to improve forage quality. The c*innamoyl CoA reductase* (*CCR*) gene was down-regulated in alfalfa using an antisense strategy. It has been shown in other plant species that the down-regulation of *CCR* results in an increased S/G ratio (lignin composition). This is thought to be due to a decrease in extractable G units, although S units are also reduced. This leads to the assumption that the *CCR* gene is involved in the synthesis of both G and S lignin. Currently,

characterization of the CCR down-regulated plant lines is being done at the phenotypic, biochemical and molecular genetic levels. The results from these studies will be presented in the poster.

08. *Kim, Jin-Hee, Soo-Un Kim, & Yung-Jin Chang.* **Expression analysis of the rice WRKY gene superfamily in response to UV-irradiation.**

WRKY proteins are plant transcription factors involved in the regulation of defense response. Recently, analyses of the rice genomic sequences have predicted 85 *OsWRKY* gene superfamily. Phytoalexins are low-molecular-weight compounds produced as part of the plant defense response. UV-irradiation of etiolated rice induces the production of phytoalexins, momilactones, and has been chosen as a convenient abiotic stress. In an effort toward understanding the complex transcriptional regulation during the activation of defense response, we systematically analyzed their expression profiles and found that a majority of *OsWRKY* genes were differentially regulated in response to UV-irradiation of etiolated rice.

09. *Kutrzeba, Lukasz, Franck E. Dayan and Jordan K. Zjawiony.* **Study on biosynthesis of salvinorin A – hallucinogen isolated from *Salvia divinorum*.**

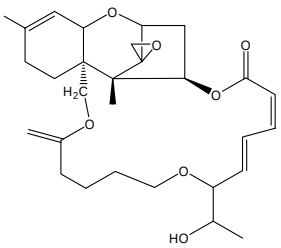
Salvinorin A is a neoclerodane diterpene found in the abaxial glandular trichomes of leaves of hallucinogenic mint *Salvia divinorum*. Having a high selectivity and affinity to □-opioid receptors, salvinorin A became a potential analgesic agent and molecular probe for competitive receptor binding assays. Its biosynthesis could be by either mevalonic acid (MVA) pathway, or 5-phospho-1-deoxy-D-xylulose (DOXP) pathway. Results from preliminary experiments, using plant cuttings, showed that neither ^{14}C-MVA nor ^{14}C-acetate were incorporated into salvinorin A suggesting its synthesis *via* DOXP pathway. To further elucidate the biosynthesis of this metabolite we used two methods. Plant cuttings were incubated with ^{13}C-DOX; incubation parameters were improved allowing for the use of 0.01-0.05% ^{13}C 1-deoxy-D-xylulose in Hoagland's medium for 8 weeks, without resulting in signs of necrosis or shock. In addition glandular trichomes were isolated and incubated with the radio-labeled geranyl pyrophosphate (^{3}H-GPP), and S-adenosylmethionine (^{14}C-SAM), in order to obtain radio-labeled salvinorin A *via* de novo synthesis. Salvinorin A extracted from both plant and glands were examined by autoradiography, HR-MS, and NMR.

10. *Michael Mazourek, David Bolliet, Charles Stewart Jr., Paul Todd Jr. and Molly Jahn.* **Where you smell smoke, there's fire: Biochemical genetics of pepper pungency.**

Capsaicinoids are the group of alkaloids that are responsible for the familiar burning sensation caused by hot peppers. Medicinally, capsaicinoids are used as the active ingredient in analgesic creams and have been shown to induce tumor cell apoptosis in vivo. Although pepper extracts are the major source of capsaicinoids for food and medicine, little is known about their biosynthesis. We have undertaken a comprehensive revision of the capsaicinoid biosynthetic pathway based on the current literature in homologous systems, differential gene expression and genetic analyses. Further, we have manipulated the capsaicinoid profile in pepper fruit by breeding peppers that stably accumulate specific and novel analogs of capsaicinoids. Some of these analog compositions may have enhanced therapeutic benefits beyond their known sensory differences. Finally, hot peppers are essential ingredients in many cuisines, but capsaicinoids themselves lack smell or flavor. We investigated the source of the piquant aroma commonly associated with spicy foods and found that the volatile compounds are synthesized in the same fruit tissue as capsaicinoids themselves. Further, accumulation of aroma compounds requires a functional allele of the BAHD acyltransferase, AT3 which is essential for capsaicinoid accumulation. We propose that the pungent aroma of hot peppers serves as an aposematic odor that functions together with capsaicinoids in the plant's defense.

11. *McCormick, Susan P., Nancy J. Alexander and Robert H. Proctor.* ***Myrothecium roridum* Tri4 controls initial three oxygenations in macrocyclic trichothecene biosynthesis.**

Macrocyclic trichothecenes are toxic sesquiterpenoids that are produced by certain fungi and plants. The unique structural features of macrocyclic trichothecenes make them 10-fold more toxic than *Fusarium* trichothecenes. *Myrothecium roridum* shares at least three trichothecene biosynthetic genes with *Fusarium: Tri5*, a sesquiterpene cyclase gene; *Tri6*, a zinc-finger transcriptional regulatory gene; and *Tri4*, a P450 oxygenase gene. *Myrothecium roridum Tri4 (MrTri4)* has a 63% identity to *F. sporotrichioides Tri4*. We recently used a heterologous expression system for trichothecene P450 genes using *Fusarium verticillioides*, a species that produces no trichothecenes, genes and found that *Fusarium Tri4* controls four oxygenation steps. In this study we expressed *MrTri4* in *F. verticillioides.*. Transgenic *F. verticillioides* expressing *MrTri4* converted exogenous trichodiene to isotrichodiol, indicating that *MrTri4* controls the initial three oxygenation steps in *Myrothecium* macrocyclic trichothecene biosynthesis.



12. *Nakashima, Jin, Fang Che.n, M.S. Srinivasa Reddy, Gail Shadle, Lisa Jackson, and Richard A. Dixon.* **Down-regulation of key enzymes in the monolignol pathway: effects on lignin composition in specific cell types of alfalfa (*Medicago sativa* L.).**

Lignin is a complex aromatic polymer resulting from the dehydrogenative polymerization of monolignols, and is the second most abundant biopolymer on earth. There is considerable interest in genetic manipulation to alter the quantity and quality of the lignin polymer for improvement of lignocellulose utilization in the areas of paper pulping, biofuel fermentation and forage digestibility. Although many genes in the monolignol pathway have now been down-regulated in transgenic plants, the effects of these manipulations on lignin polymer content and composition within specific cell types are largely unknown. We have been modifying lignin biosynthesis in alfalfa (*Medicago sativa* L.) for improvement of forage digestibility. Transgenic alfalfa lines antisense down-regulated for cinnamate 4-hydroxylase, 4-coumarate 3-hydroxylase, caffeic acid 3-*O*-methyltransferase, caffeoyl CoA 3-*O*-methyltransferase, ferulate 5-hydroxylase, or hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase were investigated in the current work. 20μm stem cross sections were made from the fifth internode from the top using a cryo-microtome, and observed under the confocal microscope for total lignin and under the light microscope after Mäule staining for S-lignin. The distribution and intensity of lignin accumulation showed significant differences between these transgenic lines. Using the above serial sections, specific cell types – vascular, fiber, and parenchyma – have been micro-dissected and measured lignin composition through thioacidolysis. This procedure allows us to address directly how the antisense genes affect lignin accumulation in each cell type.

13. *Nopo-Olazabal, Luis, Scott Simeon, Robyn Hannigan, Argelia Lorence and Fabricio Medina-Bolivar.* **Elicitation and secretion of sesquiterpenes in hairy roots cultured in the Liquid Lab™ bioreactor.**

Biotic and abiotic elicitors are efficient in stimulating production of secondary metabolites in hairy root cultures. Moreover, elicitation has been shown to induce secretion of these molecules into the culture medium reducing labor and cost associated with their purification. In order to determine the conditions for elicitation in large scale cultivation of hairy roots, we tested the simple-to-operate Liquid Lab™ (Southern Sun Biosystems, Inc.) rocker reactor for growth of *Hyoscyamus muticus* hairy roots and

production of inducible sesquiterpenes. A hairy root line of *H. muticus* which shows a color phenotype upon addition of elicitor was used to visually track the response of the roots to elicitor challenge. The elicitor was applied after weeks 2, 4 and 6 of growth and sesquiterpenes were analyzed in the medium after 24 hours by thin layer chromatography and gas chromatography-mass spectrometry. Parameters such as the age of roots before elicitation, frequency of elicitor addition, and medium exchange in the bioreactor vessel were optimized to obtain the highest yield of inducible secreted sesquiterpenes.

14. *Pang, Yongzhen, Greg Peel and Richard A. Dixon.* **Characterization and subcellular localization of *Medicago truncatula* leucoanthocyanidin reductase and anthocyanidin synthase.**

Proanthocyanidins are important branch products of the flavonoid biosynthetic pathway, and the enzymes involved in their synthesis were proposed to be localized as one or two ER-associated multi-enzymes complexes. Leucoanthocyanidin reductase (LAR) and anthocyanidin synthase (ANS) are two important enzymes leading to two different branches of the flavonoid biosynthetic pathway: catechins for the synthesis of proanthocyanidins and anthocyanidin for the formation of (epi)catechins (also leading to proanthocyanidins) and anthocyanins, respectively. In the present study, *LAR* and *ANS* genes from the model legume *Medicago truncatula* were cloned and showed high identities to their counterpart genes from other plant species. RT-PCR analysis showed that they have different expression patterns in different tissues. Transient expression in tobacco leaf epidermal cells of MtLAR fused with cyan florescent protein (CFP), and MtANS fused with yellow florescent protein (YFP), showed that both enzymes are localized in the cytosol, a finding which questions membrane-associated metabolic channeling to control the two branch pathways. Additional molecular and biochemical characteristics of the two enzymes will be presented.

15. *Peel, Gregory J., Yongzhen Pang and Richard A. Dixon.* **Detection and quantification of proanthocyanidin oligomers from transgenic plants using HPLC with post-column derivatization.**

Proanthocyanidins (PAs) are oligomeric plant compounds derived from catechin/epicatechin monomers. PAs have beneficial attributes for humans and foraging livestock. They are strong antioxidants and have been shown to benefit cardiovascular health in humans, whereas in ruminant species such as cattle and sheep, moderate levels of PAs have been shown to reduce pasture bloat and improve nitrogen utilization in the rumen. In efforts to improve the forage quality of alfalfa (*Medicago sativa*), our lab has used the model legume *Medicago truncatula* to study the impacts of over- expression of both biosynthetic (*anthocyanidin reductase*) and regulatory (*TT2*) genes. Transgenic plants ectopically expressing these genes have been previously shown to accumulate PAs; however neither the composition nor relative levels of individual oligomers have been determined. Since the composition of PAs synthesized in planta could affect forage quality, a method has been developed to rapidly quantify and determine the composition of various oligomeric PAs. The method involves normal-phase HPLC separation of semi-purified PAs followed by post-column reaction with the PA specific reagent DMACA (dimethylaminocinnamaldehyde). This procedure allows for accurate and sensitive quantitation of individual oligomeric PAs and, unlike traditional HPLC methods which rely on UV or fluorometric detection, exhaustive sample preparation and clean up are not required. Data will be shown from several transgenic materials accumulating engineered PA's.

16. *Pitta-Alvarez, Sandra I., María Alejandra Alvarez and Patricia L. Marconi.* **Hyoscyamine-6-B-hydroxylase gene: Cloning and obtention of recombinant hairy roots of *Brugmansia candida*.**

The gene that encodes for the enzyme hyoscyamine-6-β-hydroxylase was isolated and cloned from hairy roots of *Brugmansia candida*. The gene was cloned in the vector pCRTOPO2.1 and amplified by transformation with strains of *Escherichia coli* DH5α. The insert was sequenced in a DNA ABI 373 A automated sequencer (Sanger method). The isolated sequence was highly homologous to the same

enzyme derived from other *Solanaceae*. The size of the insert was 1.1 Kb, encoding a sequence of 344 amino acids. The sequenciation of the gene also showed that the initial ATG and the STOP codon were correctly located, as well as the 2OG-Fe(II) oxygenase domain. The insert was cloned in the expression vector pJIT60 under the double promoter CAMV35S. *Agrobacterium rhizogenes* LBA 9402 was transformed with this construct by triparental mating. The colonies were screened by subculture in a YMB medium with rifampycin (5 mg/L), obtaining the strain LBA-H6H. Plants of *B. candida* were obtained and cultured *in vitro* in Gamborg B5/2 medium supplemented with sucrose (20 g/L) and agar (8 g/L). They were incubated at 24 ± 1^0 C with a 16-h photoperiod. Hairy roots of *B. candida* were obtained after infection of sterile plantlets with *A. rhizogenes* strains LBA 9402 and LBA-H6H. The hairy roots that appeared at the infection sites were excised and cultured individually on B5/2 liquid medium supplemented with sucrose 15 g/l, ampicillin 2 g/l, rifampycine 5 mg/L, kanamycin 30 mg/L and agar 8 g/l. The hairy roots were incubated on a gyratory shaker at 100 rpm in the same conditions described above and were routinely sub-cultured every 2 weeks reducing 1:10 the concentration of antibiotics until the elimination of *Agrobacterium*. The induction of hairy roots from *B. candida* plants using *A. rhizogenes* LBA9402 and LBA-H6H was successful (80%). The transformation is in the process of being checked by PCR reaction.

17. *Reddy, Muntha K., Sashi K. G. Kasimsetty, Melissa R. Jacob, Shabana I. Khan and Daneel Ferreira.* **Bioactive ellagitannins from Punica granatum juice by product.**

Punica granatum L. Punicaceae, pomegranate juice by product (PJBP) (fruit husk/peel) on chromatographic separation over XAD-16 and Sephadex LH-20 columns afforded four pure ellagitannins,characterized as ellagic acid (1), gallagic acid (2), punicalin (3) and punicalagin (4). Ellagitannins (1- 4) and their XAD-16 eluated mixture of tannin fractions were evaluated for antioxidant, antiplasmodial and antimicrobial activities *in vitro*. The mixture of tannin fractions, as well as isolated ellagitannins (1- 4), exhibit strong antioxidant effects in HL-60 cells. Mixture of tannins inhibit ROS generation to 50% at concentrations of 0.8 - 19 µg/mL (IC50 values) while ellagitannins (1- 4) show IC50 values of 1.1, 3.2, 2.3 and 1.4 µM, respectively against ROS generation. All were not cytotoxic to mammalian cells up to 25 µg/mL. The mixture of tannins and pure ellagitannins (1-4) exhibit strong antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRS), *Aspergillus fumigatus* and *Mycobacterium intracellulare*. Gallagic acid (2) exhibits antiplasmodial activity against D6 and W2 strains of *P. falciparum* with IC50 values of 5.0 and 4.8 µM, respectively. This is the first study to report the antioxidant, antimicrobial and antiplasmodial activities, and SAR studies of pomegranate fruit ellagitannins 1-4. This study indicates the usefulness of PJBP and Pomegranate juice as an antioxidant, antimalarial and antimicrobial remedy in the form of dietary supplements and supports the use of pomegranate in traditional medicine.

18. *Romano, Catalina S. , Karina Abadi, María V. Repetto, Gabriel Vicchera, Adrián A.Vojnov and Silvia Moreno.* **Antimicrobial properties of Argentinean *Rosmarinus officinalis* L extracts exhibiting high antioxidant power.**

We demonstrated that rosemary plants grown in Argentine's northwest are rich sources of phenolic compounds with high antioxidant capacity. This work reports on the study of functional properties of *Rosmarinus Officinalis* extracts in order to identify the bioactive compounds responsible for their antimicrobial activity by a combination of bioassays with chemicals analysis. The interest in natural antioxidants has been increased considerably and plant antioxidants are very attractive as a natural alternative to synthetic compounds. The appearance of bacterial resistance to antimicrobial drugs (aminoglycosides, penicillin ampicillin, vancomycin, quinolones, beta-lactam) has stimulated the search for new drugs. It is clear that rosemary extract have bioactive properties according to traditional use and scientific evidence; however, the efficiency of their phenolic antioxidants has not been compared to known compounds. Our aim was to investigate the antimicrobial action of the plant extract and/or pure compound with the final objective to find synergism of the plant compounds with conventional antibiotics to inhibit pathogenic microorganisms. Our results showed that the antimicrobial rosemary extract efficacy was associated with their specific phenolic composition.

19. *Ross, Samir A., Kesanapalli S. Krishnaveni, Satoshi Takamatsu and Charles L. Burandt.* **New benzofurans and antioxidant constituents of *Zanthooxylum flavum.***

Zanthoxylum flavum Vahi. (Rutaceae), known as Espinillo (Dominican Republic), Yellow sanders (Jamaica), and Noyer Bois noyer (Guadeloupe), is an evergreen tree found in Lower Florida Keys, Bermuda, Bahamas, Cuba, Jamaica, Hispaniola, Puerto Rico, and Lesser Antilles from Anguilla to St. Luciain. *Zanthoxylum* species are reported to have many medicinal properties, and phytochemical studies on the genus have shown it to be a rich source of coumarins, lignans, and alkaloids, with febrifuge, sudorific, and diuretic properties. Previous studies on the root and stem bark of *Z. flavum* reported the isolation of three alkaloids and four coumarins. Our recent study on the roots of *Z. flavum* led to the isolation of two new benzofurans and eighteen known coumarins, sterols and alkaloids which were identified by comparison of their spectral data with literature values. Among the known compounds, fourteen compounds constitute the first report from this species. They are: bergapten, xanthotoxin, marmesin, oxypeucedanin, 6-formyl-7-methoxy coumarin, isoimperatorin, sesamin, chelerythrineacetonate, dihydrochelerythrine, norchelerythrine, skimmmianine, β-sitosterol glucoside, stigmasterol and lupeol. The compounds were assayed for their antimalarial and antioxidant activities leading to a better understanding of the chemistry and bioactivity.

20. *Ross, Samir A., Raquel Rodriguez-Guzman, Susan P. Manly and John S. Williamson.* **Xenobiotic biotransformation of 4-methoxy-n-methyl-2-quinolone isolated from *Zanthoxylum monophyllum* (P. Wilson).**

Our phytochemical evaluation of *Zanthoxylum monophyllum* (P.Wilson) has led to the isolation and identification of the alkaloid 4-methoxy-N-methyl-2-quinolone in a high yield (0.2 % w/w of total dry weight) and further bioassays indicated a significant activity of this compound against Methicillin-resistant *Staphylococcus aureus* with an IC_{50} of 1.5 μg /ml. Xenobiotic biotransformation on this alkaloid with various ATCC organisms has been conducted with the general goal of increasing the bioactivity of the compound and contribute new leads for further pharmacological research. After screening nineteen organisms, one modified alkaloid has been obtained. ATCC 9170 (*Aspergillus flavus*) and ATCC 9244 (*Cunninghamella echinulata var.echinulata*) produced the N-demethylated form, 4-methoxy-2-quinolone. The recovery of the modified metabolite was better with ATCC 9244, which gave 60% of modified alkaloid versus 20% obtained from ATCC 9170. The antibacterial activity of the alkaloid was completely lost after biotransformation. Further screenings is being performed and more results will be presented later.

21. *Ruiz, Astrith and Maria de Lourdes Tapia y Figueroa.* **Micropropagation and chromosomal determination of latex-producing *Croton* species.**

Dragon's blood is a latex extracted from several *Croton* spp. (Euphorbiaceae), including *C. draconoides*. Its usage is linked to the South American traditional medicine, particularly in promoting wound healing. A study was conducted to evaluate the *in vitro* response of buds from young latex-producing *C. draconoides* plants, to assess the acclimatization response of *in vitro Croton* seedlings and to establish the chromosomal number of selected latex producing *Croton* plants. *Croton* buds were grown in 5 different media based on Murashige and Skoog (MS) basal medium. Buds from young wine-colored latex *Croton* plants performed the best on MS supplemented with NAA, BAP, GA3 and coconut water. These plants were taller, developed more leaves and showed a higher survival rate in comparison to the red- and ochre-colored latex plants. Wine-colored latex *Croton* plants previously rooted *in vitro* were tested for acclimatization response with 4 different substrates. Plant response showed variations according to substrate composition, watering conditions (fogging), fungi control (0.1% Benlate^{TM}) and bio-stimulant application (0.1% Biogen^{TM}-1). Chromosome number for all wine-, red- and ochre-colored latex *Croton* plants was 40. Our *Croton* micropropagation method should facilitate the large scale production of this important medicinal plant.

22. *Shadle, Gail, Fang Chen, Jin Nakashima, Lisa Jackson, M.S. Srinivasa Reddy and Richard Dixon*. **Effects of down-regulation of HCT on lignin in alfalfa.**

Lignin is derived from varying proportions of three cinnamyl alcohol precursors. The two main lignin monomer types are dimethoxylated syringyl (S) units derived from sinapyl alcohol, and monomethoxylated (and thus more condensed) guiacyl (G) units derived from coniferyl alcohol. These two key monomers make up approximately 90-95% of those present in polymerized dicot lignin molecules. A third monomer type, the *p*-hydroxyphenyl or H unit, is unmethylated and is derived from *p*-coumaryl alcohol. Lignin content and/or composition correlate negatively to the efficiency of paper pulping, forage digestibility, and biofuel processing. Therefore, there is a need to generate plants which have a reduced lignin content and/or altered lignin composition. We have down-regulated the key enzymes in the lignin biosynthetic pathway in the forage legume *Medicago sativa* (alfalfa). One such enzyme that we are presently analyzing is hydroxycinnamoyl CoA:quinate/shikimate hydroxycinnamoyl transferase (HCT). HCT carries out two reactions in the lignin biosynthetic pathway. It converts 4-coumaroyl CoA to 4-coumaroyl shikimate and then converts the resulting caffeoyl shikimate, formed by hydroxylation of 4-coumaroyl shikimate, to caffeoyl CoA. Due to the position of HCT in the lignin pathway, we expected the down-regulation of its gene to have an effect on all three lignin monomers and thus on lignin content and composition. Results of the analyses thus far will be presented, and will describe overall plant phenotypes, biomass comparisons, molecular analysis of transgenics for transcript levels, cellular lignin deposition patterns, lignin thioacidolysis yields and monomer ratios, and forage digestibility.

23. *Stewart, Charles E., Jr., Michael Mazourek, Giulia Stellari, Mary O'Connell and Molly Jahn.* **Genetic and molecular analyses of the *Pun1* locus and pungency in *Capsicum*.**

The fiery hot sensation of heat, or pungency, produced in the fruit of certain pepper (*Capsicum spp.*) plants is due to the presence of capsaicin. Capsaicin is produced by the joining together of a fatty acid tail to a phenolic head group. Used as an analgesic for centuries, capsaicin is currently used to treat neurological and digestive disorders and as a chemoprotective agent during cancer treatment. Capsaicin, similar to other secondary metabolites, is taxonomically restricted, occurring only in the genus *Capsicum*. While some of the genes involved in capsaicin biosynthesis are known, regulation of this pathway is not fully understood. For nearly 500 years breeders have used the *Pun1* locus to control the presence/absence of pungency in peppers. Using a candidate gene approach we isolated the *Pun1* gene, identified it as a putative acyltransferase, and used Virus-induced gene silencing (VIGS) to establish its function in capsaicin biosynthesis. The allelic diversity of this gene was examined by sequencing *Pun1* from a diverse array of pepper genotypes. Three separate mutations of the *Pun1* gene were identified in three disparate *Capsicum* species. Complementation tests and genetic inheritance studies confirmed that these mutations were alleles of *Pun1* and that they consistently co-segregated with non-pungency. Immunolocalization of *Kas* and capsaicinoids revealed that they are expressed uniformly in the epidermal cells of the interlocular septum. The results establish that mutations at the *Pun1* locus are responsible for non-pungency in domesticated peppers.

24. *Suchocki, Piotr, Grażyna Hoser, Małgorzata Wierzbicka, Monika Dudek, Małgorzata Grzelachowska, Alicja Zobel and Mieczysław Kuraś.* **Usage of selenitetriglicerides as a potential anticancer treatment.**

The organic compound of selenium (Se^{4+}) and sunflower oil called Selol®, for which a patent was obtained 1999, is a very active scavenger of free radicals, as well as an antitumour treatment with little or no toxicity. It can be given for human clinical usage in the range of a few milligrams per kilogram of body mass, as found by Zagrodzki *et al*. (2000). Inorganic Se^{4+} as sodium selenite already showed toxicity at a concentration of 3,5 mg/kg, which in spite of promising experimental trials, is only minimally used in cancer treatment (Gasmi et al., 1997). We checked the organic selenium compound (Selol®) with the *Allium* Test, used since 1938 (Levan, 1938), as well as using a cancer cell culture HL-60, to check activity of mitosis, possible blockage of any phase of mitosis, or chromosomal aberrations.

Selol® used in different concentrations showed promising reaction and no chromosomal aberrations. We suggest its possible mode of action at the cellular level as well as in clinical testing.

25. *Nurhayat Tabanca, Betul Demirci, Kemal Husnu Can Baser, Zeki Aytac, Murat Ekici, Shabana I. Khan, Melissa R. Jacob, David E. Wedge.* **The chemical composition and antifungal activity of *Salvia macrochlamys* and *Salvia recognita* essential oils.**

Essential oils of *Salvia macrochlamys* and *S. recognita* were obtained by hydrodistillation of dried aerial parts and characterized by gas chromatography and gas chromatography – mass spectrometry. One hundred and twenty identified constituents representing 97.7% in *S. macrochlamys* and 96.4% in *S. recognita* were characterized and 1,8-cineole, borneol and camphor were identified as major components of the essential oils. The oils were evaluated for their antimalarial, antimicrobial and antifungal activities. Antifungal activity of the essential oils from both *Salvia* species was non-selective at inhibiting growth and development of reproductive stroma of the plant pathogens *Colletotrichum acutatum*, *C. fragariae*, and *C. gloeosporioides*. *S. macrochlamys* oil had good antimycobacterial activity against *Mycobacterium intracellulare* however, the oils showed no antimicrobial activity against human pathogenic bacteria or fungi up to a concentration of 200 µg/mL. *S. recognita* oil exhibited a weak antimalarial activity against *Plasmodium falciparum*.

26. *Zhao, Jianping, Rahul Pawar and Ikhlas A. Khan.* **Phytochemical investigation of *Turnera diffusa* Willd (Damiana) – A conventional herbal aphrodisiac.**

Turnera diffusa Willd., a small shrub that grows chiefly in tropical and subtropical America, was used by the ancient Mayan for treatment of giddiness and falling (loss of balance). Although many other healing properties of this plant were reported, the aphrodisiac effect has been most commonly employed as its principle use. *T. diffusa* and its variety *T. Diffusa var. aphrodisiaca* (Ward.) Urb (syn. *T. aphrodisiaca*) are generally regarded as the same plant in herbal commerce, with the popular name "damiana". The history of use of damiana is full of interesting stories, as well as controversy. Once it was said to be a marvelous aphrodisiac, and later it was said to be a hoax. In recent years, plenty of damiana-containing products regain popularity on the market, with the claims of sexual arousal and potency improvement. Surprisingly, in spite of the long history of use, the phytochemistry of damiana has not been studied extensively. Until now, there have been no sufficient evidences to substantiate the alleged function. In order to gain better insights on this conventional herb, to provide scientific bases for the authentication of the botanical sources, toxicity, as well as for further pharmacological study, we have isolated and identified 35 compounds from the leaves of *T. diffusa*. Among them, 24 compounds were first time reported for this species, including five new flavonoids. Their structures were identified by spectroscopic and spectrometric methods including 1D and 2D NMR experiments (DEPT, DQF-COSY, HMQC, HMBC, and NOESY), and ESI-HRMS analysis.