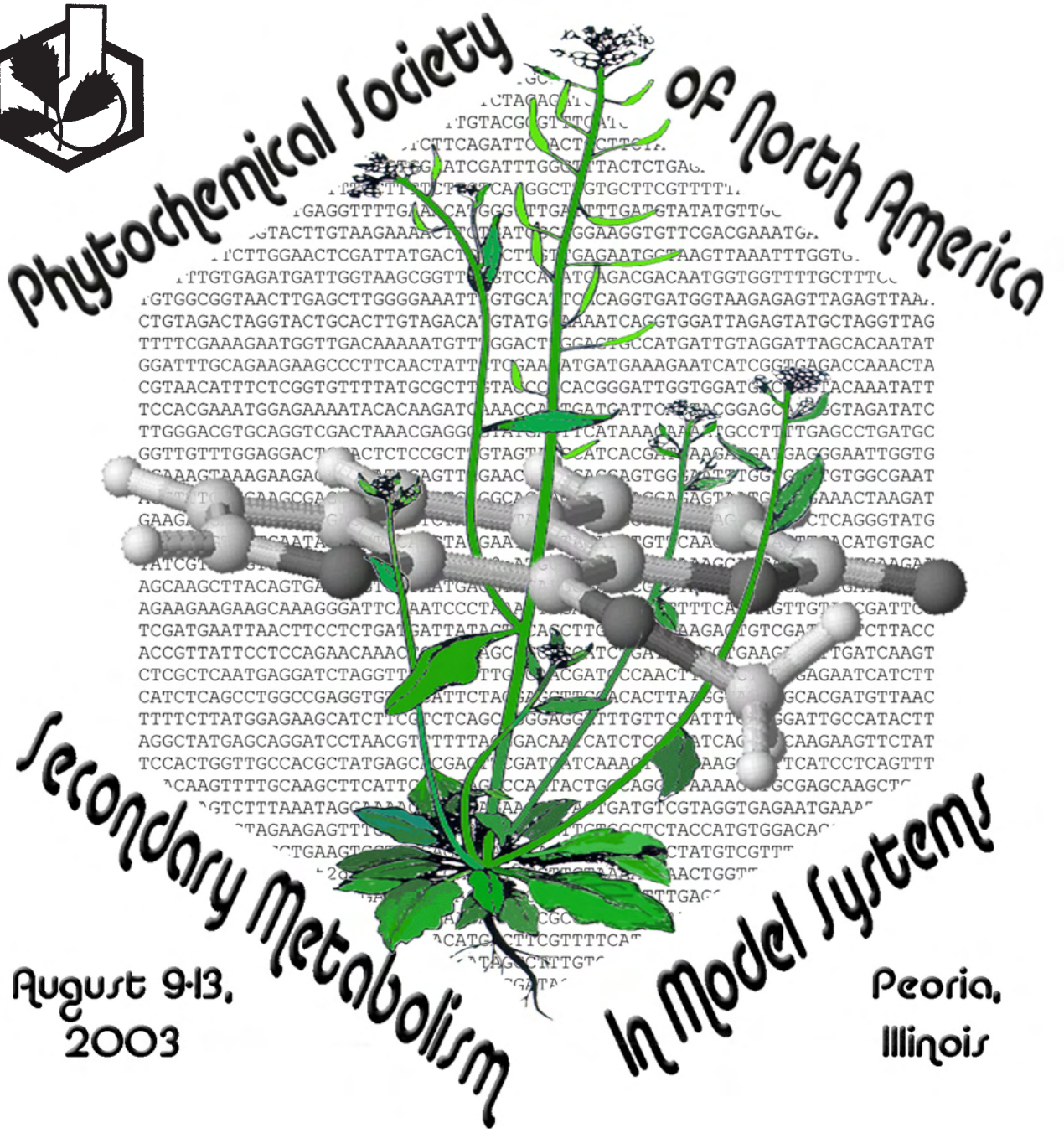


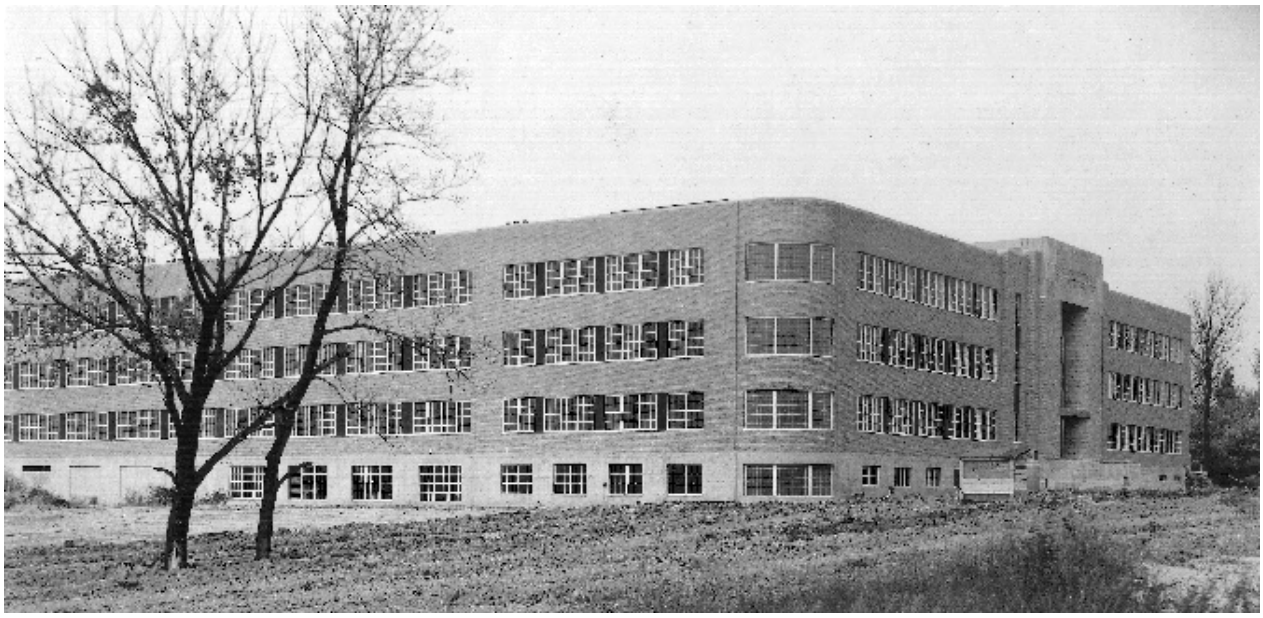
2003 CONFERENCE PROCEEDINGS

Hotel PÈRE MARQUETTE

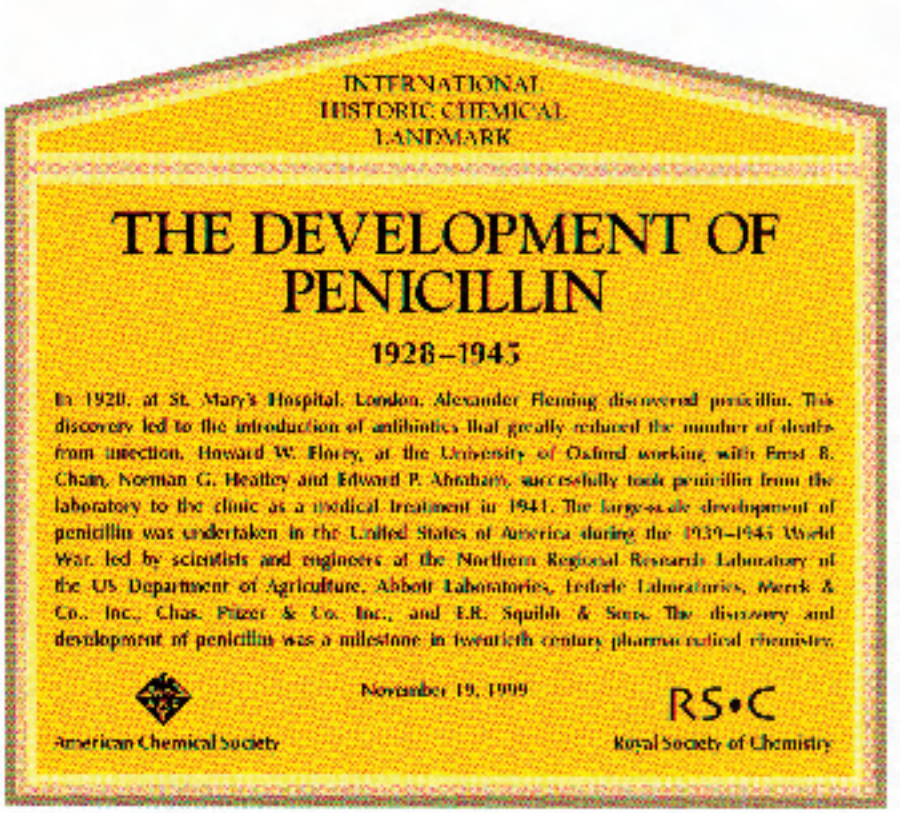
PEORIA CIVIC CENTER

USDA ARS NCAUR

Where the future is Created



Northern Regional Research Laboratory, Peoria, IL, 1938



The Phytochemical Society of North America
2003 Annual Meeting August 9-13
Secondary Metabolism in Model Systems
Peoria Convention Center, Peoria, Illinois

2003 PSNA Meeting August 9-13, Peoria, IL

Saturday August 9
12–8 pm, Registration: Hotel Pere Marquette Cotillion Room
4-6 pm, Executive Council: Hotel Pere Marquette
7–10 pm, Reception: Hotel Pere Marquette Cotillion Room

Sunday August 10
9-12 AM New Techniques Symposium, Peoria Convention Center
1-2 PM PSNA business meeting, Peoria Convention Center
2-5 PM Fungi Symposium, Peoria Convention Center
7-10 PM poster session, Peoria Convention Center

Monday August 11
8-12 AM Neish Symposium, Peoria Convention Center
12-2 PM poster session, Peoria Convention Center
6-8 PM Banquet, Peoria Convention Center

Tuesday August 12
7:30-11 AM Maize Symposium, Peoria Convention Center
11-12 AM, 1-2 PM Rice Symposium, Peoria Convention Center
2-5 PM Arabidopsis Symposium, Peoria Convention Center
6-10 PM Peoria Chiefs Baseball Game, O'Brien Field

Wednesday August 13
8-11 AM Legume Symposium, Peoria Convention Center.

Sponsor:

United States Department of Agriculture
Agricultural Research Service
National Center for Agricultural Utilization Research
1815 N. University Street
Peoria, Illinois 61604

The Phytochemical Society of North America
2003 Annual Meeting August 9-13
Secondary Metabolism in Model Systems
Peoria Convention Center, Peoria, Illinois

Sunday August 10

1 New Techniques Symposium

Chairman:

9AM **Speaker:** Dr Tom Okita
Title: Metabolic Engineering of Starch for Increased Productivity and Yields: An Integrated Approach

10AM **Speaker:** Dr Pat Wilkinsin
Title: Recent Advances in NMR: Probing the Limits of Detection

11AM **Speaker:** Dr. Mark Berhow
Title: Applied Phytochemical Analysis in the 21st Century

2-Fungi Symposium

Organizer: Dr Susan McCormick

2PM **Speaker:** Jiujiang Yu
Title: Genetics and biochemistry of aflatoxin formation and genomic approach for eliminating aflatoxin contamination

3PM **Speaker:** Dr Nancy Keller
Title: *Aspergillus nidulans:* a model system to study secondary metabolism.

4PM **Speaker:** Dr Frances Trail
Title: Form follows function in fungal sexual development

Monday August 11

3-Art Niesh Young Investigator Minisymposium

Organizer: Dr Mark Berhow

8 AM **Speaker:** Dr Eva Castells
Title: Geographical variation of poison hemlock alkaloids and its relationship with the moth *Agonopterix alstroemeriana*

8:45 AM **Speaker:** Dr John Tooker
Title: Plant defensive compounds as host plant and mate recognition cues of gall wasps

9:30AM **Speaker:** Dr Brian Traw
Title: Glucosinolates as an induced defense in Arabidopsis.

10:15 AM **Speaker:** Dr Eric Johnson
Title: Plant anthocyanin insect feeding deterrents

11:00 AM **Speaker:** Dr Benedict Hollister
Title: Host Plant Selection by Adult Colorado potato beetle: A matter of taste

The Phytochemical Society of North America
2003 Annual Meeting August 9-13
Secondary Metabolism in Model Systems
Peoria Convention Center, Peoria, Illinois

Tuesday August 12

4-Maize Symposium

Organizer: Dr Erich Grotewold

8AM **Speaker:** Dr Eleanore Wurtzel
Title: Genomics, genetics, and biochemistry of maize carotenoid biosynthesis.

9AM **Speaker:** Dr Alfons Gierl
Title: Evolution of DIMBOA Biosynthesis and Indole Production in *Zea mays.*

10AM **Speaker:** Dr Basil J. Nikolau
Title: Genetic and metabolomic analysis of epicuticular wax biosynthesis in maize

5-Rice Symposium

Organizer: Dr Tom Okita

11AM **Speaker:** Dr Mark Lange
Title: A genomic survey of metabolic pathways in rice

1PM **Speaker:** Dr Masuru Tomita
Title: Metabolomic and computational approaches to whole cell pathway modeling

***6-Arabidopsis thaliana* Symposium**

Organizer: Dr Clint Chapple

2PM **Speaker:** Dr Dorothea Tholl
Title: *Arabidopsis thaliana:* A model system for volatile terpene biosynthesis, regulation & function.

3PM **Speaker:** Dr Jim Tokuhisa
Title: Aliphatic Glucosinolate Biosynthesis in *Arabidopsis thaliana:* The evolution of an elongating story

4 PM **Speaker:** Dr Clint Chapple
Title: The Arabidopsis ref1 mutant reveals an oxidative pathway to hydroxycinnamic acids in plants

Wednesday August 13

7-Legumes Symposium

Organizer: Dr Mark Gijzen

8AM **Speaker:** Dr Richard Dixon
Title: *Medicago truncatula:* A Model Legume for the Study of Natural Products and Forage quality

9AM **Speaker**: Dr. Lila Vodkin
Title: Genetic and Molecular Control of the Flavonoid Pathway in Soybean

10AM **Speaker**: Dr. Brian McGonigle
Title: Engineering of the Soybean Phenylpropanoid Pathway for Improved Flavor & Health Benefits

Techniques Symposia 1

Metabolic Engineering of Starch for Increased Productivity and Yields: An Integrated Approach

Thomas W. Okita
Institute of Biological Chemistry, Washington State University, Pullman, WA

Plant productivity and crop yields are governed by the rate and duration of photosynthesis, the partitioning of fixed carbon between sucrose and starch in source leaves as well as the capacity of sink tissues to assimilate sugars and amino acids from source leaves. Under normal conditions, plant productivity is governed more by processes that occur in sinks which, in turn, modulate photosynthetic capacity in source leaves. This effect is clearly evident in rice where leaf photosynthesis is subject to significant "feedback" during the vegetative and heading stages of plant development suggesting a limitation in the ability to utilized photosynthate by sink organs. Many plants attempt to sustain photosynthesis when sucrose synthesis becomes saturated due to sink limitation by partitioning carbon to leaf starch. Recent studies, however, have demonstrated that this pathway is only partially effective in alleviating photosynthetic feedback as evident in Arabidopsis where the extent of leaf starch correlates with CO_2 assimilation and growth rates. In an effort to increase photoassimilate utilization and, in turn, productivity, genes that code for variants of the starch regulatory enzyme ADP-glucose pyrophosphorylase (AGPase) have been expressed in leaves during vegetative growth in Arabidopsis and rice and during rice seed development.

To modify starch production in developing rice seeds, a mutant bacterial *glgC* gene which codes for a catalytically active, allosteric effector-insensitive AGPase was utilized. The bacterial AGPase was targeted to either the cytoplasm or amyloplast, two compartments which have active AGPase enzyme activity. Transgenic rice seeds expressing the amyloplast or cytosolic AGPase-TM showed up to 11-fold higher levels of AGPase activity when assayed under inorganic phosphate-inhibited conditions compared to untransformed plants. Despite the similar levels of AGPase expression, our results indicate that the intracellular location of AGPase has a marked effect on the capacity of the enzyme to increase starch synthesis and, in turn, seed weight.

To modify starch production in leaves, a mutant AGPase large subunit was utilized. When assembled with the catalytic small subunit, the resulting enzyme displays, up-regulated properties, *i.e.* is more sensitive to 3-PGA activation and is more resistant to Pi inhibition. Transgenic Arabidopsis and rice plants have been identified that accumulate higher amounts of leaf starch. In Arabidopsis, higher leaf starch results in higher CO_2 assimilation rates due to reduced photosynthetic feedback and higher starch turnover during the diurnal cycle, translating into higher growth rates and seed yields.

Techniques Symposia 2

Recent Advances in NMR – Probing the Limits of Detection

P. Stone Wilkinson, Ph.D., Bruker Biospin Corporation, Billerica, MA *USA*

Traditional methods of identifying individual components in a mixture by NMR normally involve isolation of the components of interest. If a component is labile, it may decompose or rearrange during the isolation procedure. Multiple preparatory chromatographic separations may be necessary to provide a sufficient quantity of sample for NMR analysis in a 5mm NMR tube. Hyphenated NMR based analytical methods such as LC-NMR-MS eliminate the need for isolation of each component of a mixture. NMR, UV, and MS data may be acquired on many components within a single chromatographic run. The coupling of a solid phase extraction unit with NMR (LC-SPE-NMR-MS) can increase the amount of sample available for NMR analysis and makes the analysis of low level components of a mixture (<0.1%) possible. A summary of LC-NMR-MS and LC-SPE-NMR-MS as well as recent developments in cryogenically cooled and low volume probe technology will be presented.

Techniques Symposia 3

Applied Phtytochemical Analysis in the 21st Century

Mark A. Berhow, Steven F. Vaughn, Sandra M. Duval and Charles L. Cantrell
USDA, ARS, NCAUR, 1815 N. University St., Peoria, IL 61604

Advances in chromatography separation media, solvent delivery mechanisms, microprocessor driven hardware, computer software, and chemical detectors have combined to usher in a new era of phytochemical analysis. Technological advances have given rise to bench-top gas and liquid chromatography systems—combined with photodiode array, light scattering, and mass spectra detectors—which make the separation, analysis, identification and quantitation of chemical species in extracts prepared from plant and animal tissues fast and accurate. Simple and robust methods developed for these systems have been developed for the identification and quantitation of most secondary metabolites, such as the phenolics, terpenoids, alkaloids, glucosinolates. However, pitfalls include subtle problems with quantitation and interpretation of the non-light absorbing detectors and the lack of availability of pure phytochemicals for standards and biological evaluation. These general methods will be discussed with a number of examples, especially relating these analytical procedures to biological activity and the development of new functional agricultural products for agricultural pest control and human nutrition.

Fungi Symposia 4

Genetics and biochemistry of aflatoxin formation and genomic approach for eliminating aflatoxin contamination

Jiujiang Yu[1*], Catherine A. Whitelaw[2], Thomas E. Cleveland[1], Deepak Bhatnagar[1] and William C. Nierman[2],
[1]USDA/ARS, Southern Regional Research Center, New Orleans, LA and [2]The Institute for Genomic Research, Rockville, MD

Aflatoxins are the most carcinogenic natural toxins produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus*. The biosynthesis of aflatoxins is a multi-enzymatic process encoded by over two dozens of corresponding genes specific for these secondary metabolites in *A. flavus* and *A. parasiticus*. Studies on the molecular mechanism of aflatoxin B_1 biosynthesis have identified an aflatoxin pathway gene cluster of 70 kilobase pairs in length consisting of at least 25 identified genes including a positive regulatory gene as transcription activator in addition to the sugar and nitrogen utilization gene clusters. The completed DNA sequence of the aflatoxin gene cluster has been determined and the genes involved in aflatoxin formation have been systematically renamed from *aflA*, *aflB*, to *aflY* according to the gene convention in *Aspergillus*. In order to better understand the molecular mechanism and regulation of aflatoxin biosynthesis, plant-fungal interaction and evolutionary biology, the *Aspergillus flavus* Expressed Sequenced Tag (EST) project has been carried out at USDA/ARS, Southern Regional Research Center (SRRC). A total of 7,214 unique EST sequences have been identified from a normalized cDNA library. These ESTs represent 70%-80% total genes within the *Aspergillus flavus* genome. The *A. flavus* gene index has been constructed. Among the 7,214 unique ESTs, 3,728 tentative consensus (TC) sequences are assembled and 3,486 singleton sequences are identified from 22,324 usable sequences obtained. Microarray containing all of these unique genes (TC + singleton) has been constructed. Functional genomics studies using microarray under different conditions are underway. The application of EST/Microarray technologies will provide vital information for developing new strategies for control of aflatoxin contamination of crops.

Fungi Symposia 5

***Aspergillus nidulans:* a model system to study secondary metabolism.**

Nancy Keller
Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706

Secondary metabolites are low molecular weight natural products that are not essential to the producing cells but likely have a survival function in nature. They are of intense interest to humankind due to their pharmaceutical and/or toxic properties. We are using the model system *Aspergillus nidulans* to understand the molecular genetics of secondary metabolism in fungi. Our focus is on sterigmatocystin (ST) biosynthesis in *A. nidulans*, ST being a polyketide mycotoxin and late precursor in the aflatoxin biosynthetic pathway. Examination of ST mutants have resulted in the discovery of proteins involved in global regulation of secondary metabolism. Here we will summarize the importance of acyl CoA metabolism in polyketide production and our discovery of a global regulator, LaeA, of secondary metabolite gene clusters.

Fungi Symposia 6

Form follows function in fungal sexual development

Frances Trail
Department of Plant Biology, Michigan State University

Fungi in the Phylum Ascomycota produce their sexual spores (ascospores) in sacs called asci. In the majority of ascomycetous species, the sacs are enclosed in fruiting bodies and when mature, fire their spores into the air. We have been investigating the mechanism of forcible ascospore discharge in the cereal pathogen, *Fusarium graminearum*. *F. graminearum* produces asci inside a flask-shaped structure called a perithecium. Asci extend individually through the mouth of the perithecium and fire spores into the surrounding air. Forceful discharge of ascospores has long been speculated to be driven by turgor pressure, but the mechanism has not been elucidated. We will present evidence that accumulation of mannitol and potassium ions is important to generation of the turgor pressure for discharge of these spores. In addition, the structure of the perithecium, including wall pigments, may be important in development and function of the perithecium. The recent availability of a genomic sequence for *F. graminearum* has greatly facilited the study of perithecium development and function.

Neish Symposia 7

Geographical variation of poison hemlock alkaloids and its relationship with the moth *Agonopterix alstroemeriana*

Eva Castells, Arthur Zangerl and May Berenbaum
Department of Entomology, 320 Morrill Hall, 505 S Goodwin Ave, 61801 Urbana, IL (USA)

Poison hemlock, *Conium maculatum* L., is an herbaceous biannual originally from Eurasia and extensively naturalized in North America. All aerial parts contain piperidine alkaloids, such as coniine and γ-coniceine, which may function as chemical defenses against herbivores. Evolutionary hypotheses predict that levels of chemical defenses will vary depending on herbivory pressure. Thus, when herbivores exert sufficient selection pressure, plants will have a higher fitness when they invest in chemical defenses. In contrast, in the absence of herbivores the selective pressure for maintaining high levels of chemical defenses is relaxed and resource allocation will be focused mainly on growth and reproduction. Poison hemlock in North America was relatively free from herbivores for over a century until 1973, when it was re-associated with an important specialist herbivore from its native range, the moth *Agonopterix alstroemeriana*. This species was first found in New York State and rapidly colonized the northwestern U.S. At present, *A. alstroemeriana* is abundant and predictable on poison hemlock populations in the East and the West resulting at times in complete defoliation, whereas in the Midwest, such as in Illinois, *A. alstromeriana* is still scarce or even absent. Here, we study the variation in the production of alkaloids of poison hemlock populations under a variety of herbivory regimes. We have found differences in alkaloid concentration and composition patterns among different populations in the US and Europe consistent with some chemical defense hypotheses.

Neish Symposia 8

Plant defensive compounds as host plant and mate recognition cues of gall wasps

John F. Tooker
Department of Entomology, University of Illinois at Urbana-Champaign

Larvae of the gall wasp *Antistrophus rufus* Gillette (Hymenoptera: Cynipidae) feed within inconspicuous galls inside the flowering stems of the prairie perennials *Silphium laciniatum* L. and *S. terebinthinaceum* Jacquin (Asteraceae). Adult male *A. rufus* emerge before females and are challenged with locating mates that are sequestered within dead plant stems that occur in a matrix of dead vegetation. Allozyme studies revealed complete reproductive isolation between wasp subpopulations in the two plant species. In laboratory bioassays, males responded only to their natal plant species, antennating the stem surface. Males from *S. laciniatum* also preferred galled stems of *S. laciniatum* to ungalled stems and responded to hexane extracts of galled stems. These extracts contained much higher concentrations of monoterpenes (α-pinene, β-pinene, and camphene) than did *S. terebinthinaceum*. Ratios of "+" and "-" enantiomers of α-pinene and β-pinene approximated 50:50 for ungalled *S. laciniatum* stems but strongly differed from 50:50 in galled stems, with "+" and "-" enantiomers strongly dominant in different plants. In bioassays, male wasps from *S. laciniatum* responded to a synthetic blend of the monoterpenes in enantiomeric ratios characteristic of galled stems. Male *A. rufus* rely entirely on olfaction to locate females within stems in a complex prairie habitat, and gall wasps themselves apparently influence the plant to modify ratios of monoterpene enantiomers. These plant volatiles serve as a signal for males, serving as a sex pheromone proxy for females concealed within plant tissues.

Neish Symposia 9

Glucosinolates as an induced defense in Arabidopsis.

Brian Traw
Department of Ecology & Evolution, University of Chicago, Chicago, IL

Glucosinolates protect mustards from attack by some insect herbivores and are often induced following damage. Hormonal regulation of this induction response appears to involve both the jasmonate- and salicylate-dependent pathways. In a series of experiments, we have addressed the effects of artificial damage, jasmonic acid, and salicylic acid on the induction of glucosinolates in Arabidopsis thaliana. Artificial damage and jasmonic acid caused strong increases in glucosinolate production of leaves. The jar1-1 mutant exhibited normal glucosinolate induction following treatment with jasmonic acid suggesting that the adenylation of jasmonic acid is not required. The effect of salicylic acid depended upon the type of glucosinolate compound. Ecotypes of A. thaliana exhibited substantial differences in their response to the elicitors. Collectively, our results suggest important interactions between the two major induction pathways in the expression of these compounds.

Neish Symposia 10

Plant anthocyanin insect feeding deterrents

Eric T. Johnson and Patrick F. Dowd.
USDA, ARS, NCAUR, Crop Bioprotection Research, 1815 N. University St.
Peoria, IL 61604

The anthocyanins are synthesized by flowers primarily to attract pollinators and seed dispersers. A few studies have determined that some anthocyanin compounds can reduce insect feeding. Of several anthocyanins tested, we found that delphinidin 3-O glucoside added to insect diet at 1000 ppm significantly inhibited the growth of fall armyworms and cabbage loopers while cyanidin 3-O-glucoside only reduced the growth of fall armyworms. We also fed cabbage looper larvae leaves of an Arabidopsis thaliana transgenic mutant that accumulates cyanidin-3-O-glucosides. Leaves of the wild type control were more heavily damaged than the cyanidin-accumulating leaves. Lastly, we conducted insect feeding studies on floral tissues of Petunia hybrida, which can synthesize large amounts of different anthocyanins depending on the variety. Mortality rates of corn earworm and cabbage looper larvae were the highest on a lightly colored petunia flower that accumulates a precursor to the visible anthocyanin pigments. Together these results indicate that expression of relevant anthocyanin molecules at suitable levels may be an effective means to reduce insect predation of plant tissues.

Neish Symposia 11

Host Plant Selection by Adult Colorado potato beetle: A matter of taste

Benedict Hollister, Joesph C. Dickens and Bryan Vinyard
USDA, ARS, Henry A. Wallace Beltsville Agricultural Research Center, Plant Sciences Institute, Chemicals Affecting Insect Behavior Laboratory

Colorado potato beetle (CPB) males release an aggregation pheromone upon feeding on host potato plants. Both the pheromone and associated plant volatiles attract CPB to the host plant where the insect contacts less volatile chemicals on the surface of the plant. These phytochemicals on the surface and within the foliage are detected by sensory neurons located within gustatory sensilla on various sensory appendages of the insect including the antennae, mouthparts, and legs. Analyses of electrical activity of these neurons reveal similar response patterns for male and female CPB to several feeding stimulants: potato foliage extract, GABA and sucrose. Responses elicited by Leptine I, a glycoalkaloid feeding deterrent found in *Solanum* species, differ between the sexes. Our previous results and those presented here demonstrate the importance of the male-produced aggregation pheromone and plant volatiles in host and mate location, and indicate the involvement of nonvolatile deterrents in the final stages of host selection behaviors such as feeding and oviposition.

Maize Symposia 12

Genomics, genetics, and biochemistry of maize carotenoid biosynthesis.

Eleanore T. Wurtzel
Department of Biological Sciences, Lehman College, The City University of New York

The endosperms of cereal crops, such as maize, wheat, and rice serve as major food staple world-wide, though they are deficient in adequate levels of nutritionally essential carotenoids. In humans and animals, various carotenoids derived from plant sources act as antioxidants and protect against certain diseases, while other carotenoids are precursors to vitamin A and to retinoid compounds involved in development. Worldwide vitamin A deficiency is linked to diets deficient in pro-vitamin A carotenoids. One approach to alleviating vitamin A deficiency is to improve levels of provitamin A carotenoids in food staples such as corn, wheat, and rice by “metabolic engineering” either by marker-assisted breeding or by the use of transgenic approaches. Though pathway genes are available, there is insufficient knowledge of how the endogenous biosynthetic pathway is regulated in cereal endosperm tissue. Our present goal is to understand, at the molecular and biochemical level, how plants regulate the biosynthesis and accumulation of provitamin A carotenoids in the seed endosperm tissue. Using comparative genomics and genetics, combined with gene expression studies, we are trying to identify those factors that contribute to endosperm accumulation of carotenoids. To accomplish this basic research on the carotenoid biosynthetic pathway, we are carrying out studies in rice, which lacks endosperm carotenoids, and in maize, which does accumulate endosperm carotenoids. Use of a heterologous bacterial system has also been instrumental in testing metabolic engineering strategies, isolating new genes by complementation, and testing function of gene products. Our research will lead to better strategies for enhancing provitamin A carotenoid accumulation in endosperm tissue as well as improved understanding needed to manipulate related biosynthetic pathways. This research is currently funded by the National Institutes of Health and The City University of New York.

Maize Symposia 13

Evolution of DIMBOA Biosynthesis and Indole Production in Maize

Alfons Gierl
Lehrstuhl für Genetik, Technische Universität München

The hundreds of thousands of unique plant secondary metabolites constitute a large field of chemical biodiversity that is not only important for the survival strategies of plants but also represents an immense source for the discovery of new drugs. A secondary metabolic pathway can be defined by the branchpoint from primary metabolism and the downstream reactions that lead to end product formation. Evolution has generated specific enzymes that catalyse the branchpoint reaction and downstream processing enzymes. The biosynthesis of indole in plants branches off from tryptophan biosynthesis and is catalysed by enzymes resembling tryptophan synthase alpha subunits. In maize, indole can either function as volatile signal or be converted into benzoxazinoids by specific P450 enzymes and glycosyl transferases. These secondary metabolites function as important plant defence chemicals. Gene duplication was essential for the evolution of the genes encoding the benzoxazinoid pathway and indole formation. These evolutionary processes show that gene duplication does not necessarily result in redundany, rather it is a prerequisit for the generation of biodiversity.

Maize Symposia 14

Genetic and metabolomic analysis of cuticular wax biosynthesis in maize

Basil J. Nikolai
Iowa State University, Ames, IA 50011, USA. E-mail: dimmas@iastate.edu

A complex mixture of lipids called cuticular waxes covers the aerial portions of all terrestrial plants. As the outer barrier these lipids are important for the plant's interaction with the biotic and abiotic environment. Cuticular waxes are biosynthesized by the single layer of cells, the epidermal cells, and are secreted to the surface of the plant. To characterize the biosynthesis of this complex lipid mixture we have isolated a collection of 186 *glossy* alleles that affect normal deposition of Cuticular waxes. Allelism tests reveals that these mutants define 28 *glossy* loci, including nine previously unknown loci. HPLC and GC-MS based analyses of the cuticular waxes of maize plants have identified over 350 epicuticular wax components. The relative accumulation of these metabolites has been determined. Detailed profiling of these components among each *glossy* mutant and among four different developmental stages (seedling leaves, mature leaves, silks and pollen) and is providing clues as to the functions of each *glossy* gene. Cloning of the *glossy* genes is providing the tools for elucidating the biochemical and physiological function of each gene. For example, characterization of the cloned *glossy8* gene established that it codes for the 3-ketoacyl-CoA reductase component of the fatty acid elongase that elongates C_{18}-fatty acids to C_{20}-C_{34}-fatty acids. Elimination of this fatty acid elongation function is lethal during maize embryogenesis.

Rice Symposium 15

Genomic Survey of Metabolic Pathways in Rice

Bernd Markus Lange* & Gernot Presting

Torrey Mesa Research Institute, Syngenta Research & Development, 3115 Merryfield Row, San Diego, CA 92121 * current address for correspondence: 12580 Montellano Terrace, San Diego, CA 92130

The recent completion of a draft sequence of the rice (*Oryza sativa* L. ssp. *japonica*) genome has provided the tools necessary for an in-depth analysis of metabolic pathways related to key agronomic and quality traits. To evaluate the metabolic diversity present in rice we searched genes involved in all major plant pathways against our rice predicted peptide database. Sequence relatedness profiles were obtained for selected genes of interest to indicate the presence/absence of specific metabolic pathways across genomes. Syntenic comparisons were used to identify putative orthologs of trait-related genes in other cereals. A proteomic survey of leaf, root and grain tissues revealed the tissue-specific expression of metabolic pathways. Metabolite profiling technology was developed for the parallel analysis of multiple pathways and preliminary results obtained with different rice tissues will be presented. The integration of genomic data sets for rice gene discovery will be illustrated based on the following examples: (1) the biosynthesis of the flavor compound 2-acetyl-1-pyrroline, (2) the regulation of starch metabolism in leaves and seeds and (3) the metabolic capabilities to synthesize alkaloids.

Rice Symposia 16

Metabolomic and computational approaches to whole cell pathway modeling

Masaru Tomita
Institute for Advanced Biosciences, Keio University, 5322 Endo, Fujisawa, Kanagawa, 252-8520 Japan

In this talk, a systematic approach to constructing entire pathways of whole cell metabolism is introduced. Our approach consists of three steps: (1) Top down modeling from genomic information, (2) Bottom up modeling from metabolome analysis, and (3) Closing the gap by bioinformatics. We have developed a computer program named *GEM system* that automatically constructs whole-cell-level metabolic pathway model from genome sequence data of a given organism, based on general enzymatic information published in credible database systems, such as COG, SWISS, KEGG and EMBL. Models so constructed are incomplete due to incomplete knowledge in the published databases. To fill the gaps in the incomplete pathway models, we next identify all metabolites in the cell by CE/MS (for charged metabolites) and LC/MS (for neutral metabolites), and then bioinformatics algorithms try to connect them to complete the pathway model. The bioinformatics methods to predict unknown pathways are based on chemical structure comparisons and correlations of dynamic changes in quantity among metabolites. I will then talk about e-Rice Project, funded by the Ministry of Agriculture, Forestry and Fisheries of Japan, aiming at modeling and simulating rice metabolism using E-Cell System (http://www.e-cell.org) .

• "E-CELL: Software environment for whole cell simulation" Tomita, M., Hashimoto, K., Takahashi, K., Shimizu, T., Matsuzaki, Y., Miyoshi, F., Saito, K., Tanida, S., Yugi, K., Venter, J.C. and Hutchison, C.: Bioinformatics, 15:1, 72-84 (1999)
• "Whole cell simulation: A grand challenge of the 21st century" Tomita, M.: Trends in Biotechnology, 19:6, 205-210 (2001)
• "Towards Computer Aided Design (CAD) of Useful Microorganisms" Tomita, M.: Bioinformatics 17: 1091-1092 (2002)
• "Pressure-Assisted Capillary Electrophoresis Electrospray Ionization Mass Spectrometry for Analysis of Multivalent Anions Soga, T., Ueno, Y., Naraoka, H., Matsuda, K., Tomita, M., and Nishioka, T.; Analytical Chemistry, 74, 6224-6229 (2002)
• "Computational challenges in cell simulation: A software engineering approach" Takahashi, K., Yugi, K., Hashimoto, K., Yamada, Y., Pickett, C., and Tomita, M.: IEEE Intelligent Systems, 17:5, 64-71 (2002)

Arabidopsis Symposia 17

***Arabidopsis thaliana*: A model system for volatile terpene biosynthesis, regulation and function**

Dorothea Tholl [1,2], Feng Chen [2], John D'Auria [2], Eran Pichersky [2] and Jonathan Gershenzon [2]
1 Max-Planck Institute for Chemical Ecology, 07745 Jena, Germany
2 Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048

Terpenoids are a large group of chemicals involved in both primary and secondary metabolism in plants. In spite of their remarkable abundance and diversity, we still know little about the biological function of most terpenoids. However, the availability of genetic and genomic resources for certain model plant species provides a valuable set of new tools for exploring the physiological and ecological significance of this enormous class of natural products. We have begun to employ genetic and genomic tools to study terpene biosynthesis in *Arabidopsis thaliana* focusing on the genes of the terpene synthase family, which encode the major group of enzymes controlling the formation of terpenoid secondary metabolites. By carrying out sequence comparisons, functional characterization and gene expression studies along with profiling terpenoid metabolites, we have gained new information about the physiology and ecology of monoterpenes and sesquiterpenes in this species. The *A. thaliana* genome contains a family of 32 terpene synthase (AtTPS) genes. We have been able to show that at least 23 TPS genes are transcriptionally expressed in one or several organs (Chen, Tholl et al., Plant Cell 15:481-494, 2003). We have further showed that several AtTPS genes are expressed exclusively in flowers, and that the flowers emit a blend of monoterpenes and sesquiterpenes. Although the level of terpene emission from the flower is low compared to some strongly scented flowers such as *Clarkia breweri*, *A. thaliana* floral volatiles might nonetheless play a role in promoting outcrossing events in natural populations by attracting insect pollinators, a function now being tested in natural and synthetic populations. The occurrence of even a low level of outcrossing in this predominantly selfing species could have profound effects on its population structure and evolution. In addition to AtTPS expression in flowers, we have discovered that some AtTPS genes are induced in vegetative tissues of *A. thaliana* by herbivory and elicitor treatment, and thus might serve in a defensive role. Several additional aspects of the expression and function of AtTPSs, including their variation among ecotypes, are currently under investigation.

Aliphatic Glucosinolate Biosynthesis in *Arabidopsis thaliana:* The evolution of an elongating story

Jim Tokuhisa, Jan-Willem de Kraker, Kim Falk, Susanne Textor and Jonathan Gershenzon.
Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

Glucosinolates are secondary metabolites produced by members of the Brassicales. These compounds are considered to have important roles in plant-herbivore interactions, chemoprevention of cancer and the organoleptic properties of brassicaceous vegetables. In the model system plant *Arabidopsis thaliana*, over 20 different glucosinolates can be identified at any particular stage of the life cycle with over 35 identified among different accessions. These glucosinolates are derived from aliphatic, aromatic or indole amino acids including leucine or methionine, phenylalanine or tryptophan. As the largest known group of secondary metabolites in Arabidopsis, the glucosinolates represent an ideal system for studying the evolution and function of secondary metabolism. We are investigating the biochemical basis for glucosinolate diversity in Arabidopsis with particular attention paid to the largest group, the aliphatic glucosinolates derived from methionine. The major source of diversity resides in a pathway which introduces a methylene group in the carbon chain of methionine in a serial fashion with up to six methylene additions occuring in *A. thaliana*. We have used molecular and biochemical approches to characterize the committed step of this pathway, the condensation of an acetyl-CoA with the 2-oxo-acid derivative of methionine by a methylthioalkylmalate synthase. The recognition that this reaction is similar to one of the leucine biosynthetic pathway, led to the identification and cloning of a four-member gene family. Heterologous expression of these genes in *Escherichia coli* and the characterization of Arabidopsis lines with mutations at these loci have allowed us to assign the activities associated with leucine and glucosinolate biosynthesis to individual gene family members. The sequence similarities among these genes suggest how the reactions specific to glucosinolate biosynthesis may have evolved from primary metabolism.

Arabidopsis symposia 19

The Arabidopsis *ref1* mutant reveals an oxidative pathway to hydroxycinnamic acids in plants

Ramesh B. Nair[2], Fazeeda N. Hosein, Kristie Bastress, Max O. Ruegger[3], Jeff Denault, and Clint Chapple[1]
[1]Department of Biochemistry, Purdue University, West Lafayette IN 47907
[2]current address: Pioneer Hi-Bred International Inc. Johnston IA 50131
[3]current address: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis IN 46268

Recent work that has characterized key enzymes of the phenylpropanoid pathway has shown that the traditional view of lignin biosynthesis is incorrect. Although the hydroxylation and methylation reactions of the pathway were thought to occur at the level of the free hydroxycinnamic acids, it now seems clear that the enzymes catalyzing phenylpropanoid 3-hydroxylation and 3-*O*-methylation reactions use shikimate and CoA conjugates as substrates, respectively, and that 5-hydroxylation and 5-*O*-methylation occur at the level of the hydroxycinnamaldehydes and hydroxycinnamyl alcohols. These findings now beg the question of how sinapic acid, and possibly ferulic acid, are synthesized in plants. Recently, we have found that sinapic acid is synthesized by oxidation of sinapaldehyde, at least in Arabidopsis. This concept is directly opposite to the traditional view of phenylpropanoid metabolism in which sinapic acid is instead the precursor of sinapaldehyde. We were led to this hypothesis by the characterization of the Arabidopsis *ref1* mutant, and isolation of the *REF1* gene. The *ref1* mutant accumulates only 30% of the sinapoylmalate found in wild-type plants. Positional cloning of *REF1* revealed that it encodes an aldehyde dehydrogenase, a member of large class of $NAD(P)^+$-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding carboxylic acids. These data indicate that *REF1* encodes a sinapaldehyde dehydrogenase required for sinapic acid and sinapoylmalate biosynthesis. Importantly, when expressed in E. coli, REF1 also has coniferaldehyde dehydrogenase activity, and *ref1* mutant plants contain less cell wall-esterified ferulic acid. These findings suggest that both ferulic acid and sinapic acid are derived, at least in part, through oxidation of their corresponding aldehydes, and that manipulation of REF1 activity in plants may be a useful approach for the biotechnological modification of plants.

Legumes Symposia 20

***Medicago truncatula:* A Model Legume for the Study of Natural Products and Forage Quality**

Richard A. Dixon
Plant Biology Division, Samuel Roberts Noble Foundation, 25120 Sam Noble Parkway, Ardmore, OK 73401, USA.

Medicago truncatula (barrel medic) has emerged as model system for the study of legume biology. This species is very closely related to alfalfa (*Medicago sativa*), the world's major forage legume. Importantly, *M. truncatula* is also a bona fide forage species, and is grown extensively in Australia. Extensive EST and genomic sequence resources, metabolomic and proteomic programs, bioinformatics resources and, recently, systems for fast forward and reverse genetics, have been developed for *M. truncatula* and will be briefly reviewed. Important traits for forage crops, in addition to yield and persistence, include forage quality, digestibility, palatability, and bloating properties. Most of these traits involve secondary metabolites. Developments in genomics technology, centered on selected model species, have accelerated the pace of gene discovery in secondary metabolism and other complex pathways in plants. The rapidly emerging genomics resources for model species such *M. truncatula* will impact many aspects of forage improvement, with, in the case of *M. truncatula*, direct and immediate relevance for alfalfa. Recent progress on understanding and manipulating the biosynthetic pathways leading to lignin, condensed tannins and triterpene saponins will facilitate engineering of alfalfa and other forage legumes for reduced bloating potential and improved digestibility and palatability.

Legumes Symposia 21

Genetic and Molecular Control of the Flavonoid Pathway in Soybean

Lila Vodkin, Gracia Zabala, Jigyasa Tuteja, Steve Clough, and Wan-Ching Chan
Department of Crop Sciences, University of Illinois, Urbana, IL 61801

We describe the molecular basis of mutations affecting the flavonoid and proanthocyanin pathways in the soybean seed coat and the use of soybean microarrays to examine the expression of the pathway. Distribution of pigmentation on the seed coat is controlled by alleles of the *I* (inhibitor) locus and influenced by the *T* locus that controls color of the trichomes. The *I* locus contains a cluster of chalcone synthase (CHS) gene family members that exhibits an unusual example of naturally occurring gene silencing resulting in a non-pigmented seed coat. Seed coat proteins and mRNA are difficult to extract from black or brown pigmented seed coats with *i T* genotypes because they have procyanidins that exhibit tannin properties. We developed modified extractions methods to over come these difficulties by the inclusion of poly-L-proline in the extraction buffer to bind the procyanidins. Recently, we reported the identification of a flavonoid 3' hydroxylase gene as the *T* locus (Genetics 163: 295-309, 2003). The GmF3'H gene was highly expressed in early stages of seed coat development and very low or not at all in other tissues. GmF3'H genomic and cDNA sequence analysis of color mutant lines with varying *t* alleles revealed a frame shift mutation in one of the alleles. In another line derived from a mutable genetic stock, the abundance of the mRNAs for GmF3'H was dramatically reduced. Isolation of the *T* locus will enable investigation of its pleiotropic effects on cell wall integrity and the wavy leaf phenotype as well as its role in determining the types of flavonoids produced in various tissues and in plant defense.

Legume Symposia 22

Engineering of the soybean phenylpropanoid pathway for improved flavor and health benefits.

Brian McGonigle
DuPont Crop Genetics; E.I. du Pont de Nemours and Company

People choose to eat the foods that they do based on several criteria. Among the most important criteria are perceived health benefits, cost and taste of the food. American consumers wish to include more soy protein in their daily diets because of the health benefits attributed to soy protein. Soy protein is thought to play a positive role in preventing heart disease and hormonally related cancers, increasing women's health and potentially affecting other chronic problems of human health. Increasing the amount of soy protein as an ingredient in a variety of food products is one way for consumers to gain the benefits of soy protein. However, this approach is limited by the flavor attributes of soy protein that are not acceptable to the Western palate. Considerable effort has been applied to masking these undesirable attributes using flavoring technology or removing the "off" flavors using a variety of processing technologies with some success. However, flavor improvement remains the most important target for increasing consumer demand for soy protein products. Flavor is a complex phenomenon made up of several components including taste, color, mouthfeel and smell. We have taken a molecular genetic approach to alter the phenylpropanoid pathway of soybean to affect several of these components as well as to increase the possible health benefits from consuming soy protein.

Poster Session Abstracts

P1

GCC7, a Nuclear Regulator of Glucosinolate Accumulation in *Arabidopsis thaliana*

Maggie Levy, Qiaomei Wang, Clayton Grubb, and Steffen Abel
Department of Vegetable Crops, University of California, Davis

The cancer-preventive properties of isothiocyanates (ITCs) renewed interest in the biosynthesis and regulation of glucosinolates, plant precursors of natural ITCs. We developed a simple and efficient ITC bioassay to screen for mutants in *Arabidopsis* with altered *g*lucosinolate *c*ontent and *c*omposition and cloned the *GCC7* gene. Gain- and loss-of-function mutations in *GCC7* correlated with higher and lower glucosinolate accumulation, respectively. *GCC7* is a member of a multigene family encoding putative nuclear proteins predicted to bind to calmodulin and other EF-hand proteins. Indeed, we demonstrated nuclear localization of a GCC7::GFP fusion protein. Gene expression analysis in *gcc7* and wild type plants showed GCC7-dependent modulation of steady-state mRNA levels of several glucosinolate pathway genes. Histochemical GUS analysis of a *GCC7* gene trap line revealed spatio-temporal *GCC7* promoter activity that was strikingly similar to the expression domains observed for the two pathway genes, *CYP79F1* and *UGT74B1*. Our molecular-genetic analysis strongly suggests that *GCC7* represents a novel nuclear regulator of glucosinolate accumulation, which possibly processes Ca^{2+} signals.

P2

A functional genomics approach towards the metabolic engineering of plant natural products

Naveed Aziz[1], Gregory D. May[1], Nancy L. Paiva[2] and Richard A. Dixon[1]

[1]Plant Biology Division, SR Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402
[2]Department of Physical Sciences, Southeastern Oklahoma State University, Durant, OK 74701

We are using a functional genomics approach to investigate the enzymatic and cellular machinery involved in the production and accumulation of plant natural products in *Medicago* species. Methyl jasmonate (MeJa) has been shown to induce secondary metabolite accumulation in a variety of plant species and is suggested to be an obligate signal transduction component for elicitor-induced phytoalexin responses, irrespective of the nature of the pathway being induced. We are using gene expression profiling to identify genes induced in response to MeJa elicitation of *M. truncatula* cell cultures. In order to exploit the biosynthetic capabilities of plant trichomes for the production and storage of plant natural products, we have generated a *M. sativa* glandular trichome cDNA library and have sequenced over 3,500 clones to date. Promoter sequences will be obtained from the most highly expressed genes specifically expressed in trichomes. The promoters will be used as part of a tool kit for metabolic engineering of phytochemicals in trichomes.

P3

Synthesis and anti-microbial testing of α-pinene derivatives part II: Study of structure-activity relationship

M. Alias[1], E. Andarge[1], U. Ayala[1], P. Dhar[1], T. Snyder-Leiby[2]
Chemistry Dept.[1] and Biology Dept.[2] SUNY New Paltz

Previous work from our lab demonstrated that introducing an oxygen function to the α-pinene carbon framework (α-pinene- epoxide, α-pinandiol, α-pinan-3-ol, α-pinan-3-one), increased the anti-microbial activity of the resulting compounds. This study introduces a nitrogen function to the α-pinene carbon framework to form three nitrogen containing compounds. Product formation is monitored by TLC, IR and NMR. Antimicrobial properties of the resulting compounds against gram +, gram - bacteria, and yeast are measured by TLC agar overlay assay using MTT. Test results are compared with those obtained for α-pinene and its oxygenated derivatives to study the structure:activity relationship.

P4

Allelopathy and exotic plant invasion: from molecules and genes to communities

Harsh Pal Bais[1*], Ramarao Vepachedu[1], Simon Gilroy[2], Ragan Callaway[3], Jorge M. Vivanco[1]

[1]Department of Horticulture, Cell and Molecular Biology Graduate Program, and Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523-1173 [2]Department of Biology, Pennsylvania State University, University Park, PA 16802 [3]Division of Biological Sciences, University of Montana, Missoula, MO 59812
*Author for correspondence: Email: harsh@lamar.colostate.edu

Many of the world's most common and ecologically devastating exotic invaders competitively eliminate neighbors in invaded communities but coexist with neighbors in their native habitat. Current theories for this invasive success include the existence of empty niches in recipient communities, rapid genetic changes in invader populations in response to new selection pressures in the novel environment, special adaptation to human disturbance by invaders, and the "natural enemies" hypothesis. Here we present evidence that *Centaurea maculosa* (spotted knapweed), an invasive species in the Western U.S., displaces native plant species by exuding a phytotoxic chemical from its roots, and we provide a detailed discussion of the ecological, physiological, cellular, biochemical, and genomic mechanisms by which this type of allelopathy operates. Our results overall support a "novel weapons" hypothesis for invasive success. We identify (-)-catechin, a root secreted allelochemical, as a major factor in *C. maculosa's* overwhelming competitive dominance in many North American plant communities. Our results show inhibition of native species' growth and germination in field soils at natural concentrations of the allelochemical. This effect is due to (-)-catechin's cell-specific targeting of meristmatic and elongation zone cells in the roots of susceptible plants. Cell-specific targeting is evidenced by cytoplasmic condensation followed by a cascade of cell death proceeding backwards up through the root stele. In *Arabidopsis thaliana*, the allelochemical triggers a rapid cascade of stress responses such as the induction of a wave of reactive oxygen species (ROS) initiated at the root apex, which leads to a Ca^{2+} signaling cascade triggering a cellular pH decrease, and allelochemical-induced genome-wide changes in gene expression patterns. These responses kill cells in the root meristem and elongation zone and ultimately lead to the death of the entire root system of susceptible species.

P5

A Flavanone and Two Phenolic Acids From *Chrysanthemum morifolium* With Phytotoxic and Insect Growth Regulating Activity.

Clifford W. Beninger-1, Mamdouh Abou-Zaid-2, and Christopher J. Hall-1
1-Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada
2-Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

Leaves of *Chrysanthemum morifolium* cv. Ramat were extracted sequentially with hexane, ethyl acetate and methanol. The methanol fraction, when incorporated into artificial diet was found to significantly reduce growth of larvae of the cabbage looper (*Trichplusia ni* Hubner) at concentrations of 500-5000 PPM of diet. Fractionation of the methanol extract on a Sephadex column yielded five fractions, three of which significantly reduced weight of cabbage looper relative to the control. Fraction 4 was analyzed using high performance liquid chromatography (HPLC) and found to contain three main constituents. These three compounds were purified using a combination of Sephadex columns and HPLC and subjected to analysis by ^{1}H and ^{13}C NMR as well as chemical and physical analyses. The compounds were identified as: **1**, chlorogenic acid (5-*O*-caffeoylquinic acid), **2**, 3,5-*O*-dicaffeoylquinic acid and **3**, 3′,4′,5-trihydroxyflavanone 7-*O*-glucuronide (eriodictyol 7-O-glucuronide). At concentrations of 0.2mM-2.0 mM these compounds reduced both growth and photosynthesis of *Lemna gibba* L. with the order of efficacy being: flavanone > chlorogenic acid > 3,5-*O*-dicaffeoylquinic acid. Furthermore, when incorporated separately into artificial diet these compounds, at 0.02 and 0.2 mM,were found to significantly enhance or reduce growth of cabbage looper (*Trichoplusia ni*) and gypsy moth (*Lymantria dispar* L.) .

P6

Alkaloid Biosynthesis is Localized to Sieve Elements in Opium Poppy

David A. Bird[1], Vincent R. Franceschi[2], and Peter J. Facchini[1]*
[1]Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada; [2]School of Biological Sciences, Washington State University, Pullman, WA, 99164, USA.

Opium poppy produces an array of alkaloids including sanguinarine and morphine. Morphine is harvested from the latex of the stem, which is the cytoplasm of laticifers, cells that fuse to form an internal secretory system throughout the plant. Despite extensive studies of isolated latex, it does not appear to contain the enzymes sufficient for morphine biosynthesis, suggesting that production of alkaloids does not occur within the laticifers. To determine the location of alkaloid synthesis, we raised antibodies to three enzymes, *N*-methylcoclaurine 3'-hydroxylase (CYP80B1), which is common to both sanguinarine and morphine biosynthesis, berberine bridge enzyme (BBE), the first committed step to the production of sanguinarine, and codeinone reductase (COR), the penultimate enzyme for the production of morphine. Immunolocalization of the enzymes identified a single cell type which was found to be adjacent or proximal to laticifers, suggesting that both pathways are active within the same cells, but not in laticifers. These cells were determined to be phloem sieve elements. In situ hybridization revealed that CYP80B1, BBE, and COR mRNA were found exclusively within companion cells. Our results show that alkaloid biosynthesis does not occur in laticifers, but is instead localized within the conductive cells of the phloem. This represents the first report of an active biosynthetic pathway within sieve elements.

P7

Functional Characterization of *Medicago truncatula* Glycosyltransferases in Relation to Natural Product Biosynthesis

Jack W. Blount, Lahoucine Achnine, and Richard A. Dixon
[1]Plant Biology Division, SR Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402

Glycosyltransferases (GTs) transfer nucleotide-diphosphate-activated sugars to low molecular weight compounds, aiding in the stabilization and compartmentation 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. Using our extensive *M. truncatula* EST database, we have identified a large number of putative full length GTs which may be involved in glycosylation of secondary metabolites. These GTs will be assayed by HPLC for activity involving a number of substrates and sugars. This poster will present proof of principal for this HPLC assay method as well as the initial bioinformatics analysis of putative *M. truncatula* GTs.

P8

Benzenoid Network in Petunia flowers.

J. Boatright[1], G. Peel[1], D. Rhodes[1], C.M. Kish[1], D. Gang[2], X. L. Chen[1], and N. Dudareva[1].
[1]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907,
[2]Department of Plant Sciences, University of Arizona, Tucson, AZ

Benzenoid compounds, which determine the scent/aroma of numerous flowers/fruits, lack the three-carbon chain and originate from *trans*-cinnamic acid as a side branch of the general phenylpropanoid pathway. Formation of benzenoid compounds from cinnamic acid requires the shortening of the side chain by a C_2 unit for which several routes have been proposed. The side-chain shortening could occur via an β-oxidative pathway or via a nonoxidative side-chain cleavage with benzaldehyde (BD) as a key intermediate, or via the combination of these two pathways. We used *in vivo* stable isotope labeling and metabolic flux analysis in combination with the power of computer-assisted metabolic modeling to investigate the metabolic pathways leading to benzenoid compounds in petunia. *Petunia hybrida* cv. 'Mitchell' provides an ideal experimental system to investigate the benzenoid network, because its floral scent consists almost exclusively of benzenoid/phenylpropanoid compounds and is dominated by methylbenzoate, benzaldehyde, and phenylacetaldehyde. Petal tissue also contains substantial quantifiable endogenous intracellular pools of acetophenone, benzaldehyde, benzylbenzoate, methylbenzoate, phenylacetaldehyde, phenylethanol, isoeugenol, and eugenol. By supplying the deuterium labeled phenylalanine to excised petunia petals, a tissue highly specialized for floral scent biosynthesis, which is able to continue to emit volatile compounds after excision, we have determined the labeling kinetics of the intracellular pools of benzenoid compounds (intermediates and end products) as well as volatile end products within the floral bouquet. Computer-assisted isotopic flux analysis revealed that both benzylbenzoate and benzaldehyde are intermediates between L-Phe and benzoic acid, indicating that both pathways are involved in the formation of benzoic acid. This work is supported by USDA-NRI.

P9

Impact of elevated CO_2 or O_3 on the isoflavone content of eight soybean cultivars

Bordignon, J. Renato.; Long, Stephen P.; Engeseth, Nicki.J.
Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign

To evaluate the effects of high atmospheric concentrations of CO_2 and O_3 on soybean isoflavone content, eight cultivars of soybean were grown in a FACE (Free Air gas Concentrated Enrichment) facility at South Farms, University of Illinois. Four rings, each 20 m in diameter, were set in the field for each of the three atmospheric gas conditions evaluated, including: 1) ambient (~370 ppm) CO_2 and ambient O_3; 2) high CO_2 (550 ppm) and ambient O_3; and 3) ambient CO_2 (~370 ppm) and high O_3 (1.5 times the ambient concentration). Results indicated that atmospheric conditions affected various cultivars differently. Two cultivars of soybean, Dwight and Pana grown in elevated atmospheric CO_2 exhibited an increase in isoflavone content. Isoflavone content decreased in two cultivars, Corsoy and Dwight, under elevated atmospheric O_3. Key words: *Glycine max*, isoflavones, daidzein, genistein, FACE, HPLC.

P10

Metabolomic examination of methyl jasmonate induced *Medicago truncatula* cell cultures.

Corey Broeckling and Lloyd Sumner
Plant Biology, Samuel Roberts Noble Foundation, Ardmore, OK 73401

Medicago truncatula is emerging as a model species for economically important legumes including alfalfa, soybean, and food crops such as beans. As part of a large functional genomics project examining the proteome, transcriptome, and metabolome, we report the results of a GC/MS based metabolomic study of the response of *M. truncatula* cell culture to methyl jasmonate elicitation. This hormone has been demonstrated to be involved in secondary metabolism in *M. truncatula*. We observed increased accumulation of certain amino acids in cells treated with methyl jasmonate, including lysine and phenylalanine, the later of which serves as a precursor to phenylpropanoids such as lignin monomers and isoflavanoids. When compared to control cells, elicited cells demonstrated increased levels of beta-amyrin , a triterpene precursor to the jasmonate inducible triterpene saponins. In addition to identified metabolites, some unknown metabolites exhibit strong methyl jasmonate responses. In particular, one polar metabolite was isolated nearly exclusively from elicited cells, and continued to accumulate throughout the 48-hour time course. We will present data on elicitor-induced changes in metabolite correlations, which may be interpreted as altered enzyme activity or subcellular partitioning of metabolites.

P11

Antioxidant and Inhibitory Activities of phenoloxidases and vegetal energetic systems by secondary metabolites isolated from mexican and chilean plants.

Carlos L. Céspedes[1*], Mariana Dominguez[1], Rodrigo Salazar[1], J. Guillermo Avila[2], Patricio Torres[1], J. Camilo Marin[1] and Isao Kubo[3].
[1]Instituto de Química, [2]FES-Iztacala, UNAM, Coyoacan 04510, Mexico D.F., Mexico.
[3]Dept. Environ. Sc., Policy and Management. U. of California, Berkeley, CA, USA.

Our phytochemical studies are biodirected with aim of to find botanical origin biopesticides. We have isolated some diterpenes, limonoids, triterpenes, sesquiterpene lactones, coumarins and flavonoids from Meliaceae, Asteraceae, Celastraceae and Zygophyllaceae families and also some chemical derivatives from them. The major finding shows that these compounds, which possess plant growth regulator (PGR) and insect growth regulator (IGR) or insecticidal activities, are the natural or some derivatives compounds. The acetylated and carbonyl --unsatured derivatives showed mainly PGR activity, while, those that contain hydroxyl, carbonyl and oxy groups were more active as insecticides. The allelopathic activities were assayed in *T.pratense, L. perenne* and *P.ixocarpa* as model of weed seeds and insecticidal were assayed against the fall armyworm *(S.frugiperda*) and Mexican bean borer *(E.varivestis)* both insect pests in corn and beans, respectively the principal crops in Mexico. Very little is known about these natural compounds and its derivatives on insect and weed pests. The natural compounds that we have isolated represent a valuable resource for the dissection of their allelopathic activities and its control. Progress in the biochemical and allelopathic characterization of this pathway will be presented. This work was supported in part by UCMEXUS-CONACYT Program, CONACyT grant 27975-N and DGAPA-UNAM grant IN243802. Corresponding Author: ccespede@servidor.unam.mx, http://www.iquimica.unam.mx/cespedes.html

P12

An EST-based approach to diapocarotenoid biosynthesis in the developing seed coats of *Bixa orellana* (Bixaceae)

Colette Jako, Dauenpen Meesapyodsuk, Catherine Coutu, Ingeborg Roewer, Darwin W. Reed, Lawrence E. Pelcher, Michele Loewen and Patrick S. Covello
NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9

Bixin is a commercially important diapocarotenoid derived from the seed coats of the tropical shrub *Bixa orellana*. Based on available chemical data and by analogy to abscisic acid biosynthesis, a biosynthetic pathway for bixin starting from lycopene was proposed. This putative pathway was investigated through expressed sequence tag (EST) analysis using a subtracted cDNA library. This analysis identified ESTs corresponding to most of the enzymes of the carotenoid biosynthesis pathway including the 1-deoxy-D-xylulose 5-phosphate pathway of plastids. ESTs corresponding to a number of genes encoding dioxygenases and carboxyl methyltransferases were also identified. Progress in identifying genes specific

P13

Metabolic engineering of isoflavonoid biosynthesis in alfalfa

B.E. Deavours and R.A. Dixon
Plant Biology Division, SR Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402

Isoflavones such as genistein have generated considerable interest as nutriceuticals and epidemiological and animal model studies have linked dietary consumption of genistein and high soy diets with lower instances of cancer, improvement of cardiovascular disease, and alleviation of post-menopausal symptoms. Isoflavones are naturally limited to legumes (major dietary sources are soy products), but isoflavone synthesis can be engineered into other plants by introduction of the enzyme isoflavone synthase (IFS). The ability to engineer isoflavone synthesis into food crops provides an excellent test case for determining the value of dietary delivery of health beneficial compounds. In order to better address the effects of dietary isoflavones on health, we have engineered genistein production in alfalfa by constitutive expression of IFS. Over 50 independent transgenic plants were analyzed and genistein levels in the leaves of these plants ranged from 0-150 nmol/g FW. These plants will be used to determine the effect of plant-derived isoflavones on animal health using an animal model system such as rats, which are commonly fed alfalfa pellets.

P14

Functional Divergence of Transcription Factors and Metabolic Diversity

Anusha P Dias and Erich Grotewold
Department of Plant Biology & Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210

R2R3 MYB domains are a family of transcription factors widely distributed in plants. We have characterized a group of *R2R3 Myb* genes (R2R3 Myb^{PtoA}) that has amplified recently (past 50 million years) in the grasses. R2R3 Myb^{PtoA} is characterized by at least 10 members, including the well characterized maize *P* gene. *P* regulates the accumulation of 3-deoxy flavonoids, insecticidal *C*-glycosyl flavones and phlobaphene pigments found only in a few grass species, supporting the hypothesis associating recent expansion of *R2R3 Myb* genes and metabolic diversity between related plant species. To further investigate the evolution of regulatory mechanisms that have resulted in novel metabolic pathways, we studied *ZmMyb-IF35*, another R2R3 Myb^{PtoA}. We used a gain of function approach in maize Black Mexican Sweet (BMS) to determine the function of *ZmMyb-IF35*. Metabolite profiling of maize cell lines overexpressing *ZmMyb-IF35* shows the accumulation of ferulic and chlorogenic acids. Mapping of *ZmMyb-IF35* close to a minor QTL for chlorogenic acid in maize silks strongly suggest that *ZmMyb-IF35* encodes a novel regulator of the accumulation of phenolic compounds in maize. Our studies validate the application of metabolite profiling to establish the functions of plant transcription factors, and provide insights on how novel plant metabolic pathways may have evolved.

P15

Mapping the limits of the *TRI4/TRI6* bidirectional promoter in *Fusarium graminearum* and analysis of its transcription factor binding sites

Rex B. Dyer, David F. Kendra and Daren W. Brown
Mycotoxin Research Unit, USDA/ARS/NCAUR, Peoria, Illinois 61604

Fusarium graminearum, the causative agent of wheat scab, is an international problem due to economic losses to the agricultural industry and because of food safety concerns resulting from mycotoxin contamination. Mycotoxin synthesis is coordinated by the *tri6* gene which encodes a transcription factor. In order to better understand the regulation of *tri6* gene expression and mycotoxin synthesis, we have employed the method of 5'-RACE to map the limits of the bidirectional promoter within the *tri4/tri6* intergenic region of *Fusarium graminearum* strain GZ3639. We conclude that this bidirectional promoter consists of 892 nucleotides, mapping from -109 to -1000 nucleotides 5' of the *tri4* translational start codon. We analyzed the DNA sequence of this bidirectional promoter and the analogous sequence from 41 other *Fusarium* strains for transcription factor binding sites. Our analysis of the 42 strains reveals 21 to 30 transcription factor binding sites, specific to 14 different transcription factors. The transcription factor binding site that is most commonly represented in each strain is specific to the Nit2 transcription factor. The Nit2/Area family of transcription factors upregulate genes involved in the utilization of secondary nitrogen sources when primary nitrogen sources become limiting. This nitrogen regulatory circuit is known as nitrogen metabolite repression. We hypothesize that the *nit2* sites within the *Fusarium graminearum tri4/tri6* bidirectional promoter function to bring *tri4* and *tri6* gene expression, and mycotoxin biosynthesis under nitrogen metabolite repression control.

P16

Biochemistry, Molecular Genetics and Genomics of Volatile Biosynthesis in Arabidopsis

Feng Chen[1], Dorothea Tholl[1,2], John C. D'Auria[1], Afgan Farooq[2], Jeannine R. Ross[2], Joseph P. Noel[3], Jonathan Gershenzon[2], and Eran Pichersky[1]
[1]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA; [2]Max Planck Institute for Chemical Ecology, Beutenberg Campus, Winzerlaer Strasse 10, D-07745, Jena, Germany; [3]Structural Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

Arabidopsis is emerging as a model system to study the biochemical, physiological, and ecological functions of plant volatiles. Under normal growth conditions, Arabidopsis plants emit a blend of volatiles, including terpenoids from the flowers and medium-chain aldehydes from the leaves. In contrast, herbivory and elicitor treatments induce the emission of novel volatiles, of which methyl salicylate (MeSA) is a prominent one. A multidisciplinary approach that included bioinformatics, expression profiling, metabolite profiling, and biochemical genomics was adopted to identify genes encoding the enzymes responsible for volatile biosynthesis in Arabidopsis. Two gene families, the AtTPS terpene synthases and the AtSABATH methyl transferases, potentially involved in the biosynthesis of terpenoids and methyl esters, respectively, were identified in the Arabidopsis genome using *in silico* sequence analyses. Tissue-specific and induction-specific volatile emission from Arabidopsis plants was determined by headspace analysis. The expression of all AtTPS and AtSABATH genes was examined by RT-PCR. The biochemical function of specific AtTPS and AtSABATH genes was determined by cDNA expression in *E. coli* followed by *in vitro* enzyme assays. Here we show how this methodology has led to the identification of a leaf-inducible AtSABATH gene that encodes an enzyme that can catalyze the synthesis of both methylsalicylate and methylbenzoate.

P17

In silico analysis of serine carboxypeptidase-like proteins in _Arabidopsis thaliana_.

Christopher M. Fraser[1], Amber M. Shirley[2], Mitsuyasu Hasebe[3] and Clint Chapple[1]
[1]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA; [2]BASF Plant Science L.L.C., 26 Davis Drive, Research Triangle Park, NC 27707, USA; [3]National Institute for Basic Biology, Nishigonaka 38, Myodaiji, Okazaki 444-8585 Aichi, Japan

The *Arabidopsis thaliana* genome includes a family of 53 genes encoding proteins similar to serine carboxypeptidases, a class of enzymes generally assigned to roles in protein processing and turnover. Two of these serine carboxypeptidase-like (SCPL) proteins have now been identified as glucose acyltransferases involved in sinapate ester production, reflecting a catalytic activity distinct from that implied by annotation. A preliminary phylogenetic analysis has grouped these two SCPL proteins and 17 others from *Arabidopsis* together with three glucose acyltransferases from *Solanum berthaultii* and one from *Lycopersicon pennellii*. In an attempt to clarify the structural and functional characteristics shared by these proteins, we present an *in silico* analysis using freely available bioinformatics software such as ClustalW, Phylip, NNPredict, GenTHREADER, ProQ, SignalP and PSORT. This project is intended to serve as an hypothesis-generating step in the complete examination of SCPL proteins in *Arabidopsis*, an undertaking that may ultimately provide insight into the catalytic divergence of enzymes and the evolution of plant secondary metabolism.

P18

Light induces the _de novo_ activation of root phenylpropanoid metabolism in Arabidopsis

Matthew R. Hemm and Clint Chapple
Department of Biochemistry, Purdue University, West Lafayette IN 47907

Phenylpropanoid biosynthesis is less well studied in roots than in aerial tissues. Previous work with reporter gene constructs has shown that *C4H* and other phenylpropanoid genes are highly expressed not only in lignifying root vascular tissue but also in nonlignifying tissues such as the root cortex, suggesting that these genes are involved in other aspects of phenylpropanoid metabolism. We have found that although few phenylpropanoids can be found in two week-old soil-grown Arabidopsis roots, exposure to light induces the accumulation of high levels of many soluble phenylpropanoids, including coniferin and syringin (coniferyl and sinapyl alcohol-4-*O*-glycosides) as well as a number of flavonoids. The biosynthesis of these compounds appears to be a light-specific response, since their accumulation is not induced in dark-grown seedlings when exposed to chemical elicitors that induce phenylpropanoid metabolism in other tissues, such as methyl jasmonate, salicylate and ABA. Biochemical analysis of light-response mutants shows that PHYA and PHYB are the primary photoreceptors involved in the accumulation of coniferin and syringin, and that HY5 activity is required for the phenylpropanoid accumulation response. One possible explanation for this phenomenon is that these metabolites serve to protect exposed roots from stresses potentially associated with light exposure in nature, such as UV damage, desiccation and pathogen attack. These results also suggest that Arabidopsis roots may offer a novel system for investigating the regulation of phenylpropanoid biosynthesis.

P19

Estimated clearance rates for simmondsin metabolites

Ronald A. Holser[1], Maurits Van Boven[2] , and Marnix M. Cokelaere[3]

[1]USDA-ARS-NCAUR,Peoria, Illinois, 61604 USA
[2]Laboratory of Toxicology, Katholieke Universiteit Leuven, Belgium
[3]Interdisciplinary Research Center, Katholieke Universiteit Kortrijk, Belgium

The jojoba plant, *Simmondsia chinensis,* is indigenous to the Southwest American desert and is cultivated for the wax esters in the seed. The plant also produces a group of bioactive glycosides, i. e., simmondsin, simmondsin ferulate, demethyl simmondsin, and didemethylsimmondsin, that elicits an anorexic response in mammals after ingestion. These glycosides are distributed throughout the plants tissues and may protect the plant from predation by herbivores. Simmondsin, 2-cyanomethylene-3 hydroxy 4, 5 dimethoxycyclohexyl β D glycoside, has been studied in controlled feeding trials with Wistar rats. A two-compartment model was applied to dose-response data to describe the absorption of simmondsin and estimate the clearance rates of the aglycan in rats fed 5 mg simmondsin/g meal over a four-hour period.

P20

The *Arabidopsis ref1* mutant reveals an alternative pathway to hydroxycinnamic acids in plants

Fazeeda N. Hosein, Ramesh B. Nair[2], Kristie Bastress, Max O. Ruegger[3], Jeff Denault, Clint Chapple[1]
[1]Department of Biochemistry, Purdue University, West Lafayette IN 47907
[2]Pioneer Hi-Bred International Inc. Johnston IA 50131
[3]Dow AgroSciences LLC, 9330 Zionville Road, Indianapolis IN 46268

Arabidopsis and other members of the Brassica family accumulate sinapoylmalate in their leaves and sinapoylcholine in their seeds, both of which fluoresce under UV light. The *ref1* mutant was identified by its reduced epidermal fluorescence when observed under UV light, a phenotype that can be attributed to the lower sinapate ester content of its leaves. The *REF1* locus was mapped to chromosome 3 and positional cloning revealed it to encode an aldehyde dehydrogenase of the ALDH2 family which has sinapaldehyde dehydrogenase (SALDH) activity. Biochemical analysis of the heterologously expressed REF1 protein also revealed that the enzyme has coniferaldehyde dehydrogenase activity (CALDH). To evaluate the relevance of this latter activity *in vivo,* we made a *ref1* x *fah1*double mutant. HPLC analysis revealed that *ref1* is epistatic to *fah1* for the production of feruloylmalate, a compound accumulated at low levels in the *fah1* mutant in place of sinapoylmalate. These data demonstrate a role for REF1 in the formation of ferulic acid, the precursor for feruloylmalate. Further, HPLC analysis of cell wall hydrolysates revealed that cell wall-linked ferulate esters were reduced to 50% of wild type, providing further evidence that REF1 is needed for ferulic acid synthesis. These data indicate that it is necessary to reassess conventional views of how hydroxycinnamic acids are made in plants.

P21

Biosynthesis of Decursin in *Angelica gigas* Nakai

Xiuhong Ji, Bum Huh and Soo-Un Kim
School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

To establish biosynthetic pathway of decursin in *Angelica gigas*, umbelliferone and decursinol were labeled with deuterium. These deuterated compounds and other commercially available putative precursors (L-phenylalanine-*ring*-d_5, *trans*-cinnamic acid-d_7) were fed to the hairy root culture of the plant. It was shown that each deuterated compound was incorporated into decursinol, and decursinol angelate or decursin. These findings confirmed the pathway deduced from individual steps found in other plants was also operating in *A. gigas*. Acknowledgment: Supported by Plant Diversity Research Center and Plant Metabolism Research Center.

P22

Characterization of Acyltransferases Involved in Phenylpropanoid Metabolism

Sarah C. Hunter[1], Christine M. Kish[2], Irina Orlova[2], Natalia Dudareva[2], Maria del Carmen Ramirez[3], David R. Gang[1]
[1]Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA. [2]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA; [3]College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA.

Several acyltransferases (hydroxycinnamoyl transferases, HCTs) catalyze reactions at branch points in the phenylpropanoid pathway. *p*-Coumaroyl-CoA shikimate transferase (CST) forms *p*-coumaroyl-5-*O*-shikimate, an important intermediate in the general phenylpropanoid pathway leading to lignins, sinapoylmalate, and eugenol. Other HCTs (*p*-coumaroyl-CoA quinate transferase, CQT, and *p*-coumaroyl-CoA 4-hydroxyphenyllactate transferase, CPLT) form intermediates in the branches of the phenylpropanoid pathway leading to chlorogenic and rosmarinic acids, respectively. HCTs have been identified in a number of plants, including apple, *Arabidopsis*, basil, date, ginger, petunia, tobacco and turmeric. HCTs from different plants have different substrate preferences. For example, basil acyltransferases demonstrate high substrate specificity: only shikimate serves as acyl acceptor and only *p*-coumaroyl-CoA as acyl donor for basil CST. Basil CPLT is highly specific for 4-hydroxyphenyllactate as acyl acceptor, although it transfers *p*-coumaroyl and caffeoyl moieties with similar efficiency. Basil CST and CPLT are distinct enzymes. Basil does not possess CQT activity. In contrast, *Arabidopsis*, ginger, tobacco and turmeric possess both CST and CQT activities, but no CPLT activity. The ratio of CST to CQT activity varies in ginger and turmeric, depending on the tissue, suggesting two different enzymes, although a tobacco HCT was recently reported to possess both activities. Genes encoding acyltransferases from a number of these plant species have been isolated and are being characterized.

P23

Purification and Characterization of Geraniol Synthase from the Peltate Glands of Sweet Basil (*Basilicum ocimum*)

Yoko Iijima[1], Efraim Lewinsohn[2], and Eran Pichersky[1]
[1]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, [2]Department of Vegetable Crops, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay 30095, Israel

Geraniol is a simple acyclic monoterpene alcohol emitted from flowers of many species. High levels of geraniol synthase (GES) specific activity were found in the peltate glands of the lemon-scented sweet basil cultivar Sweet Dani, which contains mostly citral (geranial and neral) as the principle monoterpene constituents but also some geraniol and nerol. GES was purified from the glands of this basil cultivar using a series of chromatographic steps and characterized. GES uses geranyl pyrophosphate as the precursor, and geraniol is the exclusive reaction product. It requires manganese ion as a divalent metal cofactor for activity. GC/MS analysis of geraniol produced by the GES catalysis in a reaction solution containing ^{18}O-labeled water showed that the hydroxyl group of geraniol came from water, suggesting that GES uses a reaction mechanism similar to that of other terpene synthases and distinct from that of phosphatases.

P24

AN IMPRESSIVE RANGE OF C-GLYCOSYLFLAVONES IN THE FERN PTERIS VITTATA

Filippo Imperato
Dipartimento di Chimica dell'Università della Basilicata, 85100 Potenza, Italy.

Luteolin 6-C-rhamnoside-8-C-arabinoside (1), luteolin 6-C-glucoside-8-C-arabinoside (2) and an apigenin-di-C-pentoside (3) have been isolated from aerial parts of the fern Pteris vittata L. These three C-glycosylflavones and four C-glycosylflavones previously reported from this fern (3, 8-di-C-arabinosylluteolin, luteolin-6-C-arabino- side-8-C-glucoside, 3-C-(6'''-O-acetyl-b-cellobiosyl)-apigenin and 6-C-cellobiosyl- isoscutellarein 8-methyl ether) show that Pteris vittata has an impressive range of C-glycosylflavones; in Pteridophyta a similar situation has been found previously in the fern Angiopteris evecta (Marattiaceae) but this species lacks flavonoid O-glycosides . which are present in Pteris vittata. Flavonoid (1) is a new natural product. The author thanks SESMA (CNR, Naples) for mass spectra measurements.

P25

A NEW FLAVONOL GLYCOSIDE FROM THE FERN PTERIS VITTATA

Filippo Imperato
Dipartimento di Chimica dell'Università della Basilicata , 85100 Potenza, Italy.

Two flavonol glycosides (1 and 2) have been isolated from aerial parts of the fern Pteris vittata L. by preparative paper chromatography followed by Sephadex LH-20 column chromatography. Flavonoids 1 and 2 have been identified as kaempferol 3-O-arabinopyranosyl-(1 2)-rhamnopyranoside and quercetin 3- O-sophoroside respectively by chemical and spectral methods. Flavonoid 1 is a new natural product whereas flavonoid 2 is a new fern constituent. The author thanks SESMA (CNR, Naples) for mass spectra measurements.

P26

Chalcone Isomerase: More than Just an Enzyme?

Niloufer G. Irani and Erich Grotewold
Department of Plant Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210

Chalcone isomerase (CHI), a central enzyme of the flavonoid/isoflavonoid pathway, cyclises chalcones to flavanones, a reaction known to occur spontaneously *in vitro*. Maize *35S::R+C1* BMS cell lines constitutively accumulating anthocyanins, show no detectable levels of CHI mRNA or activity. In *Arabidopsis*, there is total dependence on the enzyme, as *tt5* (CHI) produce no flavonoid. These contrasting observations prompted us to further investigate of the function of CHI. Similar to the other steps in the pathway, the monocot enzyme complements *Arabidopsis tt5*. To establish whether the catalytic activity of CHI is required for this complementation, we mutated the highly conserved, catalytically important residues Y104F, T46A and N34A in the maize protein. *Zm*CHI^{Y104F}, with ~20% of the wild type maize CHI activity, has the ability to complement *Arabidopsis tt5* with no obvious change in the quantitative or qualitative levels of anthocyanins. Complementation and analysis of *Arabidopsis tt5* with the single, double and triple mutants of the afore mentioned three residues is currently under progress. Limited activity of the mutated enzyme, with a fully functional pathway, promotes the provocative idea of a 'moonlighting' role for CHI. Chalcone isomerase may not only be catalytically active , but may provide a structural hold for the enzymes involved in the various branches of the pathway. This would ensure efficient partitioning of metabolites, depending on the need of the environmentally challenged plant.

P27

Light and Anthocyanins: Beyond Gene Regulation

Niloufer G. Irani and Erich Grotewold
Department of Plant Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210

The induction of the flavonoid biosynthetic pathway by light is mediated by the increased expression of the regulators. Therefore, constitutive expression of the regulators should not alter the accumulation of anthocyanins in response to light. However, anthocyanin pigmented maize Black Mexican Sweet cells expressing the MYB and HLH regulators, C1 and R, from a light insensitive *35S CaMV* promoter (35S: :C1+R), showed visual darkening when exposed to high light. Interestingly, there was no increase in steady state mRNA levels for CHS (*c2* gene) or DFR (*a1* gene), or in the quantitative accumulation of the anthocyanins. *In vivo* reflectance measurements (CIELab values) showed an unexpected "yellowing" of the light grown calli, as compared to the dark grown cells. These results are indicative of an additional level of control of the pigmentation properties of the anthocyanins. The physiological and cellular effects of light on 35S:: C1+R maize BMS cells are currently under investigation and will be discussed.

P28

A reverse genetics approach to lignin modification in *Medicago*

Lisa Jackson, Gail Shadle, M.S. Srinivasa Reddy, Fang Chen and Richard A. Dixon
Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK, 73401, USA.

Lignin is a complex polymer formed by the oxidative polymerization of hydroxycinnamyl alcohol derivatives termed monolignols, and imparts mechanical strength to plant stems and hydrophobicity to water conducting vascular elements. Because of the impact of lignin on forage quality and paper pulping efficiency, there is a considerable interest in genetic manipulation to modify the quantity and/or quality of the lignin polymer. *Medicago truncatula* (barrel medic) is a forage legume with a simple diploid genome and is very closely related to the world's major forage legume, *Medicago sativa* (alfalfa) that has a complex genome. Genes from *M. truncatula* share very high sequence identity to their counterparts from alfalfa making *M. truncatula* an excellent model for understanding the molecular biology of alfalfa. We have utilized *M. truncatula* as a model species for a genomics-based approach to understanding monolignol biosynthesis. To date, transgenic approaches to lignin modification (by antisense and cosuppression strategies) have not taken into account the potential complexity of the gene families being targeted. We want to exploit the recent advances in plant gene silencing technology based on an understanding of RNA-interference to facilitate the molecular dissection of the functions of individual members of the lignin pathway gene families, and this may allow for more precise engineering of lignin biosynthesis. We will describe:
1. Assessment of RNAi using tobacco as a rapidly transformable model system.
2. Gene complexity in the *M. truncatula* monolignol pathway.
3. Initial results of transformation of *Medicago* species for lignin modification.

P29

Herbivory Alters the Spatial Pattern of Photosynthesis and Gene Expression in *Arabidopsis thaliana*

Jennie Tang, Raymond Zielinski, Arthur Zangerl, Antony Crofts, May Berenbaum, Evan DeLucia
Department of Entomology, 320 Morrill Hall, 505 S Goodwin Ave, 61801 Urbana, IL (USA)

Effects of herbivory by first instar *Trichoplusia ni* on photosynthesis and expression of an *Arabidopsis thaliana* Rubisco small subunit gene within a leaf were measured in transgenic plants through fluorescence measurements of chlorophyll and green fluorescent protein, respectively. We observed a strong negative effect of herbivory on the remaining foliage—a further 11% reduction in photosynthesis in addition to the 19% loss by removal of leaf tissue. Within a damaged leaf, there were two types of photosynthetic damage caused by herbivory: minor depressions in photosynthesis immediately around the rim of holes and major photosynthetic reduction in areas well beyond the holes that spread over time. There was spread of photosynthetic damage in some leaves and not in others. Herbivory caused a 26-57% reduction in photosynthesis in some parts of the damaged leaf. It also stimulated photosynthesis in un-damaged areas of the same leaf by 3%. Parallel decreases in GFP fluorescence suggest that herbivory reduced expression of the Rubisco small subunit gene and may have contributed to the reduction in photosynthesis. Alternatively, water stress induced by nicking of veins by herbivores may have reduced photosynthesis. The practice of equating the potential loss of carbon gain with the amount of leaf tissue removed may thus underestimate the impact of herbivory.

P30

Semiochemicals at the leaf surface: The composition of celery *(Apium graveolens)* epicuticular waxes

Stefanie Schaffer, Tanja Schleicher, Reinhard Jetter
Depts. of Botany and Chemistry, University of British Columbia, Vancouver, Canada

At the very surface of plants 'epicuticular waxes' form a thin film, inevitably acting as a substratum for herbivores and pathogens when they first contact the tissue. Employing new methods for selective probing and chemical analysis, we found that jasmonate induction triggers the accumulation of furanocoumarins at the surface of celery leaves. The subsequent evaporative loss of furanocoumarins from the surface film was monitored, hence allowing a complete assessment of their turnover kinetics. The composition of the surrounding surface wax mixture was not changed by jasmonate treatment or by evaporation. The epicuticular film was extremely thin and differed in its chemical composition significantly from the underlying intracuticular compartment. We hypothesize that the transient (spatial) accumulation of furanocoumarins allows direct pathogen defense, while (temporally) restricting host recognition by herbivorous insects.

P31

Triterpenoids at the plant surface: Slippery grounds for insects

Christian Kinzler, Ortwin Guhling, Reinhard Jetter.
Depts. of Botany and Chemistry, University of British Columbia, Vancouver, Canada

Stem surfaces of the ant-plants in the genus *Macaranga* (Euphorbiaceae) are covered with threadlike crystals. They reduce the adhesive force of insect feet and hence limit insect mobility on the plant surface, representing a mechanical barrier to walking insects. Our comparative analyses showed that the presence of crystals on *Macaranga* surfaces is correlated with the accumulation of specific triterpenoids. These compounds, when purified and recrystallized *in vitro,* yielded crystals with shapes and arrangement similar to those on authentic plant surfaces. In conclusion, *Macaranga* surface crystals consist of specific triterpenoids, mainly epitaraxerol and taraxerone. Weare currently cloning relevant triterpenol synthase cDNAs, in order to obtain the tools for future investigations into the spatial and temporal expression patterns of corresponding enzymes.

P32

Determination and Comparison of Pyrrolizidine Alkaloids from the Genus *Omphalodes*

Ron B. Kelley, Torrissa J. Wood, and Mandi L. Conrad
Department of Chemistry, Eastern Oregon University, La Grande, OR 97850

As part of a continuing chemosystematic study of natural products from the plant family Boraginaceae, pyrrolizidine alkaloids (PA's) are studied for their biological effects. Pyrrolizidine alkaloids are potentially toxic secondary products commonly found in the family Boraginacae, and their occurrence in the genus *Omphalodes* is to be expected. In previous research on *Omphalodes verna* with TLC and GC-MS, two undetermined PAs arose. Based on this previous research, further investigation will be done in an attempt to determine the structures of these PAs through NMR analysis. A GC-MS analysis of PA profiles from other species of *Omphalodes* will be used to generate a chemosystematic based comparison.

P33

Characterization of Naphthazarins and Pyrrolizidine Alkaloids found in *Lithospermum multiflorum*

Richard D. Champion, Margaret F. Auble, Dean R. Kuenzi, Bina J. Hall, and Ron B. Kelley
Department of Chemistry, Eastern Oregon University, La Grande, OR 97850

As part of the ongoing study of naphthazarin and pyrrolizidine alkaloid compounds found in the plant family Boraginaceae, we attempted to qualitatively and quantitatively characterize these compounds from *Lithospermum multiflorum. Lithospermum multiflorum*, found in southwestern North America, is known to possess reddish root dyes indicative of the presence of naphthazarin type compounds. The naphthazarin quinoid compounds have been shown to have beneficial antibiotic and wound healing properties. Pyrrolizidine alkaloids are toxic secondary natural products also commonly found in the Boraginaceae and certain subfamilies of the Asteraceae. Two novel compounds that are likely derivatives of arnebinone, tentatively named demethoxyarnebinone and lithospermone, have been identified and partially elucidated using high-speed countercurrent chromatography, GC-MS, and NMR. Other known naphthoquinones and benzoquinones that have been identified using GC-MS include arnebinone, arnebinol, isovalerylshikonin, and 2'-methylbutyrylshikonin. In contrast to previous root dye studies of the genus *Lithospermum*, benzoquinones were found in far greater amounts in comparison to naphthoquinones. The major pyrrolizidine alkaloids found were lithosenine and macromerine.

P34

Fungal Fractions Stimulate Loblolly Pine Growth, Morphogenesis and Secondary Metabolism

Naseem I. Khan [(1)], and Brent Tisserat [(2)]
[(1)] Department of Biology, Bradley University, Peoria, IL [(2)] Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, U. S. Department of Agriculture, Agricultural Research Service, Peoria, IL.

Pinus taeda L., loblolly pine, is a valuable and widely cultivated timber and pulpwood tree grown primarily in the southern United States. Several million loblolly pine seedlings are produced annually from commercial nursery facilities. Enhancement of seedling growth and differentiation will reduce the nursery time and speed overall field establishment. Fungal elicitors are known to enhance secondary metabolism and sometimes growth. We sought to determine the value of various fungal mycelium cultures to improve pine plant growth. Loblolly pine seedlings grown in greenhouse were exposed a variety of fungal species and isolates. A number of different fungal fractions were tested. All fractions types influenced fresh weights, leave numbers, root numbers, and shoot length over control ($P = 0.05$). For example, loblolly pine seedlings exposed to certain fungal elicitors exhibited increased fresh weight over 100 % compared to untreated controls. Monoterpene levels, e.g., α-pinene, were similar for all treatments. However, overall total monoterpenes were greater in fungal treated seedlings due to their increased biomass compared to non-treated seedlings. Fungal elicitors appear to have merit in improving pine growth, differentiation and secondary metabolism.

P35

Analysis of full-length cDNA library from *Ginkgo biloba* and expression patterns of MEP and ginkgolide pathway genes and P450 gene

Sang-Min Kim[1], Kwang-Il Lee[1], Yung-Jin Chang[1], Back Hie Nam[2,3], and Soo-Un Kim[1,2]
[1]School of Agricultural biotechnology, Seoul National University, Korea
[2]Plant Metabolism Research Center, KyungHee University, Korea
[3]Department of Biological Science, Myongji University, Korea

Using 'oligo capped' mRNA and □Zap cDNA construction method, we constructed full-length cDNA library from callus and embryo root of *G. biloba*. Among them, 69 % of callus and 75 % of root had homology with the reported genes with known function. Singletons of root and callus were found to be 760 and 740, respectively. Time-course expression patterns of several MEP and ginkgolide pathway genes and P450 gene (with unknown function) in *G. biloba* embryo were analyzed by real-time PCR. As expected, the levels of expression of MEP-related genes were higher in root than in leaf, reflecting the biosynthesis of ginkgolide in root. Acknowledgments: Supported by Plant Diversity Research Center and Plant Metabolism Research Center.

P36

Metabolite Profiling as a Tool for Identification of Phytochemicals Associated with Host-Plant Resistance against Insect Pests

Brian D. McGarvey and Cindy A. Buelow
Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON N5V 4T3

A method is presented for identifying plant secondary metabolites associated with host-plant resistance against insect pests. Leaf extracts of several cultivars of florist's chrysanthemum, which varied in their degree of resistance against the western flower thrips (*Frankliniella occidentalis*), were analyzed using GC-MS. Mean areas for each of ~100 peaks in chromatograms of 5 replicates of each cultivar were obtained. The mean peak areas for a given peak from each cultivar were tested for correlation with the resistance rank for the respective cultivar. Several compounds exhibited correlations ($p < 0.01$), indicating a potential association with resistance against thrips. Tentative identifications were made based on library searches of mass spectra.

P37

Metabolic engineering of essential oil biosynthesis in peppermint

Soheil S. Mahmoud and Rodney Croteau
Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

We have manipulated the expression of several monoterpene synthases in transgenic peppermint, which has been developed as a model for the study of monoterpenoid metabolism, in order to elucidate regulation of isoprenoid biosynthesis, and to improve the yield and quality of the essential oil (mostly monoterpenes) in this agronomically important species. Our findings indicate that the expression of some of the monoterpene biosynthetic genes (e.g., menthofuran synthase) is primarily controlled at the level of transcription, and that overexpression of these genes improves metabolic flux through the corresponding pathway steps. However, the expression of other genes (e.g., pulegone reductase) is posttranscriptionally regulated; in these cases, ectopic expression fails to improve flux and leads to gene silencing. Metabolic engineering has led to increased essential oil yield, and decreased menthofuran and pulegone (both undesired oil constituents) content. In other plants, downstream biosynthetic steps were suppressed to increase limonene concentration in the oil. Furthermore, a novel "feed-forward" regulatory role for the monoterpene end product menthofuran has been discovered.

P38

Ginsenosides as Allelopathic Agents

Robert W. Nicol[1] Lina Yousef[1], James A. Traquair[2] and Mark A. Bernards[1]
[1]Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7 and [2]SCPFRC, Agriculture and Agri-Food Canada, London, ON, Canada, N5V 4T3

Ginsenosides are triterpenoid saponins found in the roots of *Panax* species such as American ginseng (*Panax quinquefolius*). They are prized for their purported medicinal value and have long been used as adaptogens. It is unlikely, however, that *Panax* species make ginsenosides for the benefit of humans, so we asked the question: what function do ginsenosides have in the ecology of *P. quinquefolius*? Previously we demonstrated that the growth of some soilborne fungi was inhibited *in vitro*, while that of others was stimulated, when ginsenosides were incorporated into their growth medium (Nicol et al, 2002, Can. J. Bot. 80:557-562). These data suggested that if ginsenosides were present in the rhizosphere at a sufficient concentration to be biologically active they might act as allelopathic agents. To address this question, ginsenosides were isolated from soil associated with the roots of commercially grown American ginseng, identified via LC-MS and quantified via analytical HPLC. The isolated ginsenosides (F_{11}, Rb_1, Rb_2, Rc, Rd, Re and Rg_1) averaged 0.06% (w/w) of the mass of the soil. To investigate whether this concentration of ginsenosides was sufficient to affect fungal growth, bioassays were performed using ecologically relevant (i.e. 0.06% w/w) concentrations of purified compounds. Thus, after addition of an ecologically relevant concentration of purified ginsenosides to the culture medium, the growth of *Pythium irregulare* (an important ginseng root pathogen) was significantly stimulated whereas that of *Trichoderma hamatum* (a saprotrophic fungus) was slightly (but not significantly) inhibited. These results confirm that ginsenosides are present in the soil at bioactive concentrations, supporting a role for them as allelopathic agents in the rhizosphere.

P39

Geranyl Diphosphate Synthase: Cloning, Characterization, and Its Involvement in the Regulation of Monoterpene Formation in Snapdragon Flowers.

Irina Orlova[a,1], Dorothea Tholl[b,1], Christine M. Kish[a], Jonathan Gershenzon[b], and Natalia Dudareva[a,2].
[a] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA and [b] Max Plank Institute for Chemical Ecology, Beutenberg Campus, Winzerlaer Strasse 10, D-007745 Jena, Germany. [1] These authors contributed equally to this work.

Snapdragon floral scent is dominated by myrcene and (*E*)-β-ocimene, for which geranyl diphosphate (GPP, C_{10}) is the immediate precursor. Using a functional genomics approach, we isolated two identical cDNA clones from a snapdragon petal-specific library that have 53% amino acid sequence identity to the small subunit of a previously isolated GPP synthase from peppermint. Expression of this cDNA in *Escherichia coli* yielded no detectable prenyltransferase activity. However, when this cDNA was coexpressed with the *Mentha piperita* GPPS large subunit, an active GPPS was obtained. This suggested the heterodimeric subunit architecture of GPPS in snapdragon. A search of snapdragon EST database and a screening of the snapdragon petal-specific cDNA library with the peppermint large subunit of GPPS resulted in the isolation of two cDNA clones (GGPPS1 and GGPPS2) with 75% and 41% of amino acid sequence identity to the large subunit of GPPS from peppermint, respectively. Coexpression in *E. coli* of the small subunit of GPPS with GGPPS1 yielded a functional heterodimer that catalyzed the synthesis of GPP as a main product and GGPP as a minor product, demonstrating that the small subunit is capable of interacting with a second prenyltransferase protein to make GPP. Analyses of tissue-specific, developmental, and rhythmic expression of the small subunit of GPPS, GGPPS1, and GGPPS2 in snapdragon revealed that the expression of the small subunit closely correlates with monoterpene emission, indicating that it might play a key role in regulating the formation of GPP, and thus monoterpene biosynthesis. This work is supported by NSF grant MCB-0212802.

P40

A microarray-based approach to study the lignin biosynthesis pathway in *Medicago truncatula*

M.S. Srinivasa Reddy and Richard A. Dixon
Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73402

The examination of global changes in gene expression using microarrays can provide insights into tissue- and developmental-specific expression of genes and the response of gene expression to various stimuli. In our studies we have used alfalfa (*Medicago sativa*) and *Medicago truncatula* (*a* model legume) to study the phenylpropanoid pathway with special emphasis on lignin biosynthesis. Since lignin biosynthesis is not yet clearly understood, a functional genomics approach could provide some of the answers as changes in gene expression underlie many biological phenomena. Oligonucleotide arrays have been generated using selected EST sequences of *M. truncatula*. Differential gene expression has been studied in young and old stem tissue in *M. truncatula* to look at the developmental regulation of lignin biosynthesis. As a result of high gene sequence homology between *M. truncatula* and *M. sativa*, we have successfully used the same arrays for *M. sativa*. Initial results show developmental differences in the expression profiles of genes involved in lignin biosynthesis. We are also comparing expression profiling of caffeic acid 3-O-methyltransferase (COMT) and caffeoyl CoA 3-O-methyltransferase (CCOMT) down-regulated transgenic *M. sativa* with vector transformed control plants. Expression profiling is also being done for elicited cell cultures of *M. truncatula* using the oligonucleotide arrays. Results from all these experiments will be presented.

P41

_p_-Coumaroyltyramine as a Mediator of Hydrogen Peroxide Production During Suberization

Fawzi A. Razem and Mark A. Bernards
Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7

Wounding of potato tubers triggers the generation of reactive oxygen species (ROS) and the formation of a wound-induced, suberized periderm. We recently demonstrated that hydrogen peroxide is required for the polymerization of the poly(phenolic) domain of suberin (Razem and Bernards, 2002, J. Agric. Food Chem. 50:1009-1015), and that the most likely source of hydrogen peroxide for suberization is a plasma membrane NADPH-dependent oxidase, immuno-related to that of the human phagocyte (Razem and Bernards, 2003, J. Ex. Bot. 54:935-941). However, the production of ROS post wounding occurs in two phases: first, there is an initial burst immediately after injury followed by a series of larger oscillations of ROS release, apparently in association with wound healing. Unlike the initial burst, or first phase, the production of ROS during wound healing (i.e., the second phase) is dependent on new protein synthesis. The oscillations in ROS generation in suberized tissues post-wounding could be partially corrected *in vitro* by treatment with hydroxycinnamic acids and derivatives, particularly *p*-coumaroyltyramine (which itself accumulates in response to wounding). That is, plasma membrane preparations with low oxidase activity levels in *in vitro* reconstitution assays could be restored to peak activity levels with the addition of *p*-coumaroyltyramine. Similarly, the inhibitory effect of staurosporine, a protein kinase inhibitor, on ROS generation in *in vitro* reconstitution assays could be compensated for by treatment with *p*-coumaroyltyramine. It is concluded that the production of ROS in association with suberization is under tight regulatory control and endogenous metabolites, particularly *p*-coumaroyltyramine, are likely involved in the regulation.

P42

Antimicrobial Bioassays for Crude Medicinal Plant Extracts - A Critical Evaluation. Isolation, Partial Purification and Characterization of *Streptomyces Akiyoshiensis* L-138 N-acetyltransferase.

Rocío C. Rodríguez-Juarez and Kevin C. Smith
Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada

Streptomycetes are considered exceptionally well-endowed microorganisms capable of generating numerous bio-active secondary metabolites. Many different enzymes are involved in the biosynthesis of these diverse metabolites. We have found that *Streptomyces akiyoshiensis* produces the natural product N-acetyl-L-DOPA. The primary focus of this study was to isolate, purify and characterize this N-acetyltransferase (NAT) in order to understand its function. This protein was isolated from liquid cultures of *S. akiyoshiensis* L-138. NAT was isolated from cell free extracts using Cibacron Blue F3G-A affinity chromatography, anion exchange chromatography and gel permeation chromatography. We are reporting the partial purification of a protein with a high specificity for L-DOPA. The characterization of this protein will be reported in terms of pH and temperature optima, effect of metal ions, substrate specificity, and kinetic parameters.

P43

Structural and Functional Studies of the SABATH Family of Plant Methyltransferases

Jeannine Ross[1], Chloe Zubieta[1,2], Feng Chen[3], Yue Yang[3], John D'Auria[4], Eran Pichersky[3], and Joeseph P. Noel[1]
[1]Structural Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037
[2]European Molecular Biology Laboratory, 38042 Grenoble Cedex 9, France
[3]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109
[4]Max Planck Institute for Chemical Ecology, Beutenberg Campus, Winzerlaer Strasse 10, D-07745, Jena, Germany

The SABATH (salicylic acid, benzoic acid, theobromine synthase) family of plant methyltransferases (MTs) is a diverse group of enzymes that methylate either the carboxyl or nitrogen functionality of their substrates, forming a methyl ester product. We have solved the 3.0 Å crystal structure of the *Clarkia breweri* salicylic acid MT (SAMT), and used this structure to do molecular modeling of several other family members. Using active site mutagenesis and substrate specificity assays, we are exploring the functional differences between the plant SAMTs, the *Arabidopsis* JMT, the recently discovered auxin MT, and the uncharacterized SABATH family members.

P44

Phytochemical Compounds and Genetics in Actinidia, Malus and Vaccinium.

DD Rowan, JM Allen, H Boldingh, C Deng, EN Friel, EA MacRae, AJ Matich, M McNeilage and M Wang.
HortResearch, Private Bag 11030, Palmerston North, New Zealand.

Phytochemical profiles for specific apple, kiwifruit, and blueberry tissues have been measured and assembled into a compound database. Similar tissues have been used to make over 120 EST libraries that have been sequenced to depths of 500 to 10,000 EST's. Our focus has been on the biosynthesis of flavour and fragrance compounds with 212 terpenes (37 of unknown structure) and 241 esters (6 unknowns) so far identified. Biosynthetic pathways for these compounds are being tested through in vitro and in vivo approaches.

P45

Isolation and Pathway Elucidation of Phytotoxins from *Phoma pomorum* - A Potential Biocontrol Agent Against *Cynoglossum officinale* L.

Kevin Smith[1], Stephen Hoare[1], Wesley Prepchuk[1] and Robert Conner[2],
[1]Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada, and
[2]Agriculture and Agri-food Canada, Lethbridge, Alberta, Canada.

The weed *Cynoglossum officinale* L. is a potential threat to range land utilization in western Canada and the United States. Recent efforts to develop methods of biocontrol have led to the isolation of the fungi *Phoma pomorum* from leaf lesions collected in the field. We have been able to elucidate the structures of three naphthalenone phytotoxins and recent efforts have focused on elucidating their biosynthesis. We will report on current results from stable isotope feeding studies and the use of the fungicide inhibitor, tricyclazole, in efforts to shed light on this pathway.

P46

Tissue-specific expression of berberine biosynthetic genes and alkaloid accumulation in *Thalictrum flavum*, a medicinal member of the Ranunculaceae

Samanani N and Facchini PJ
Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada

Berberine is a benzylisoquinoline alkaloid with potent antimicrobial properties, which suggest it functions in the defence of plants against pathogen challenge. In the meadow rue (*Thalictrum flavum* ssp. *glaucum*), berberine was found to accumulate in specific tissues as the major alkaloid. In the rhizome, protoberberine alkaloids were detected throughout the pith and, to a lesser extent, the cortex, but were absent from the vascular tissues. In the roots, alkaloid accumulation was detected only in the endodermis at the onset of secondary growth and, subsequently, in the pericycle cells of older roots near the base of the stem. Berberine biosynthesis begins with the condensation of two tyrosine derivatives to form norcoclaurine. Eight additional enzymes convert norcoclaurine to berberine. Northern blot analysis showed that seven berberine biosynthetic gene transcripts are most abundant in the roots and rhizome, which are also the primary sites of alkaloid accumulation. In the rhizome, *in situ* RNA hybridization analysis showed that the expression of these biosynthetic genes primarily occurred in the epidermis of young leaves and in shoot meristems, whereas expression in the roots was restricted to the endodermis and adjacent cortical cells. The tissue-specific biosynthesis of berberine in *T. flavum* will be discussed in relation to the localization of alkaloid pathways in other plant species.

P47

CLONING PUTATIVE GLUCOSYLTRANSFERASE (GT) GENES FROM GRAPEFRUIT SEEDLINGS: SEARCHING FOR 7GT.

Sibhatu, M.B., Pike, L.M., and McIntosh, C.A.,
Dept. Bio. Sci., Box 70703, East Tenn. State Univ., Johnson City, TN 37614.

Flavonoids are one of the most abundant classes of plant secondary products. Most are present in glycosylated form. UDP-glucose is frequently used as sugar donor and reactions are catalyzed by UDP-glucose: GTs. Flavanone-specific 7GT involved in production of bitter naringin in grapefruit has been purified and characterized from leaves. It has some unique properties, but its structure is unknown. Our objective was to obtain GT cDNA sequences from which amino acid sequences could be inferred. Gene Specific Primers (GSPs) were designed from the Plant Secondary Product Glucosyltransferase (PSPG) box, a consensus sequence of the UDP-glucose binding domain of GTs. A cDNA library was produced from young grapefruit leaf RNA using the SMART RACE RT-PCR system and the GSP-rev primer used to amplify 5' regions of GTs. Several bands were isolated and 4 distinct 5' clones were obtained. To obtain information on the total sequence, clone specific primers (GSPs) were designed from each 5' clone and used in 3'RACE PCR reactions. We have generated two putative complete sequences by overlapping information from the 5' and 3' clones; the other two 5'cDNA clones are being finished. We present sequence analysis results.

P48

Antimicrobial Bioassays for Crude Medicinal Plant Extracts - A Critical Evaluation

Crystal Snyder and Kevin C. Smith, Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada.

Agar diffusion and broth dilution techniques are the most common methods used in the screening of novel antimicrobial agents. These methods are very effective for water soluble, highly purified antibiotics such as those encountered in clinical microbiology but they are often unsuitable for screening non-aqueous crude plant extracts. These methods lack the sensitivity required for detecting antimicrobial activity in crude medicinal plant extracts and tend to be too time-consuming for screening large numbers of extracts against multiple organisms during bioassay-guided purification. Here we evaluate the most common diffusion and dilution methods, including disk diffusion, agar well, dilution with tetrazolium indicators, as well as TLC bioautography and fluorescence based assays such as those using fluorescein diacetate or alamarBlue_ for use in screening medicinal plant extracts for antimicrobial activity.

P 49

Phenotypic Analysis and Preliminary Mapping of the Arabidopsis *ref4* Mutant

Jake Stout[1], Max Rugger[2], Ethan Romero-Severson[1], and Clint Chapple[1]
[1]Department of Biochemistry, Purdue University, West Lafayette IN 47907
[2]Dow AgroSciences LLC, 9330 Zionville Road, Indianapolis IN 46268

The phenylpropanoid pathway produces many secondary metabolites that are important in plant metabolism, development, and defense. Primary products generated via this pathway in *Arabidopsis* include flavonoids, lignin monomers, and hydroxycinnamic acid esters. Sinapoylmalate is the primary phenylpropanoid metabolite accumulated in leaves. Because this compound fluoresces under ultraviolet light, mutations in genes required for this pathway exhibit a *reduced epidermal florescence* (*ref*) phenotype. Of these mutants, *ref4* has not yet been characterized in depth. Developmentally, the *ref4* mutants are dwarfed plants with slightly spatulate shaped leaves. Leaf sinapoylmalate levels range from 29% (*ref4-1*) to 16% (*ref4-3*) of wild-type levels. Furthermore, *ref4* seeds are lighter in color, indicating that the *ref4* mutation perturbs flavonoid biosynthesis. Lignin profiles of these mutants indicate that these mutants deposit less lignin than the wild-type, but exhibit no change in the ratio of guiacyl to syringyl lignin subunits. In light of these data, our current working hypothesis is that the *ref4* mutants are defective in a positive transcriptional activator of one or more phenylpropanoid pathway genes. The ref4 locus has been mapped to a 214 kb interval at the bottom arm of chromosome 2.

P50

Carbon levels influence rosmarinic acid levels in tissue cultures of *Mentha spicata L.*

Brent Tisserat and Mark Berhow
USDA, ARS, NCAUR, Fermentation Biochemistry Technology Research, New Crops and Production Research, 1815 N. University Street, Peoria, IL 61604

Technical Abstract: Rosmarinic acid is constitutively expressed in spearmint (*Mentha spicata* L.) grown in vitro. However, high positive correlations occur between carbon levels and spearmint plantlet growth (fresh weight), morphogenesis (leaves, roots, and shoots), and rosmarinic acid concentrations (mg rosmarinic acid/g dry weight). Spearmint shoots were grown under 350, 1,500, 3,000, 10,000 or 30,000 µmol mol^{-1} CO_2 for 8 wks on a Murashige and Skoog medium containing 3% sucrose under a 16-h (day)/8-h (light) photoperiod at a light intensity of 180 umol s^{-1} m^{-2}. Increased levels of CO_2 produced increased growth, morphogenesis, and rosmarinic acid concentrations. For example, plantlets grown in 350 µmol mol^{-1} CO_2 produced 5.2 ± 1.19 mg rosmarinic acid/g dry weight while plantlets grown under 30,000 µmol mol^{-1} CO_2 produced 14.0 ± 2.03 mg rosmarinic acid/g dry weight. In other experiments, the influence of media carbon on spearmint shoots growth, morphogenesis, and secondary metabolism was tested. Spearmint shoots were grown under 0.0, 0.3, 1.0, 3.0, and 5.0% sucrose, glucose, or fructose under ambient CO_2. Regardless of the carbohydrate type, increasing the carbohydrate levels resulted in a corresponding increase in rosmarinic acid concentrations. However, growth and morphogenesis increased proportionally with the carbohydrate levels to reach a maximum at 3%, and these responses declined thereafter. These results suggest that high plant growth and morphogenesis and secondary metabolism can all occur simultaneously in vitro. Carbon levels supplied by either the nutrient medium or by the atmosphere significantly influenced rosmarinic acid concentrations.

P51

Isolation and Purification of Nitriles and Isothiocyanates, Products of Glucosinolate Degradation

Steven F. Vaughn* and Mark A. Berhow; USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604

The crucifer family, Brassicaceae, is an economically important family for its many food crops. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates, which with their degradation products are primarily resonsible for the characteristic aromas and flavors of crucifers. Glucosinolate degradation products include substituted isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidinethiones, which vary depending on the plant species studied, side-chain substitution, cell pH and cell iron concentration. Many of these degradation products have biological activity ranging from insect attractants to suspected human health benefits, but are not commercially available. We have developed methods for isolation and purification of several of these degradation products, including the isothiocyanates erucin, iberin, cheirolin, hesperin, and lesquerellin (as well as most of the corresponding nitriles) using seed from several different genera within the Brassicaceae as the source of parent glucosinolates. By manipulating seed source, reaction conditions (e.g. temperature, pH) and through solvent partitioning, relatively pure (>95%) compounds can be isolated without necessitating any chromatographic separation.

P52

Root specific elicitation and exudation of fluorescent β-carbolines in transformed root cultures of *Oxalis tuberosa*

Ramarao Vepachedu, Harsh Pal Bais, Jorege M Vivanco
Department of Horticulture and Landscape Architecture, Colorado State Universtiy, Fort Collins, Colorado-80523

Stable transformation was achieved in oca (*Oxalis tuberosa* L.) using an *Agrobacterium rhizogenes*-mediated system. Transformation frequencies varied with the use of different types of strains of *A. rhizogenes* and age of explants. The transfer of *rol A* into the oca genome was confirmed by PCR analysis. In vitro transformed root cultures of oca grown in sterile liquid media induced purplish-blue fluorescence of the culture flask medium when irradiated with UV light. We have previously observed a similar phenomenon, the exudation of the fluorescent compounds by roots of in vitro and field grown oca plants. Hairy root cultures of *O. tuberosa* transformed with *A. rhizogenes* (ATCC-15834) exuded constitutive levels of harmine (7-methoxy-1-methyl-β-carboline) and harmaline (3,4-dihydroharmine), the main fluorescent compounds detected from oca's root exudates. Transformed roots showed better growth and exudation of harmine and harmaline compared to the untransformed normal roots. Upon elicitation with fungal cell wall elicitors from *Phytopthora cinnamoni*, the production and exudation of harmine/harmaline was enhanced in both transformed and non-transformed roots. Harmine and harmaline showed a wide range of antimicrobial activity against soil-borne microorganisms. Biologically, these findings suggest that in nature β-carbolines are constitutive antimicrobial compounds released into the rhizosphere upon microbial challenge. Transformed root cultures of oca makes a simple, reliable and well defined model system to investigate the molecular and metabolic exudation of fluorescent β-carboline biosynthesis, and to evaluate the biological significance of the phenomenon of root exudation of fluorescent metabolites.

P 53

Directed Metabolite Profiling: Everything Old is New Again

Weaver LM, Kurtzweil M, Chott B, CaJacob C, Gruys K
Monsanto Company, 800 N Lindbergh, St. Louis, MO 63167

In plants, compounds derived from isoprenoids have a wide variety of physiological roles: hormones, defense compounds, antioxidants, and pigments. An important class of isoprenoid compounds, the carotenoids, act as antioxidants that help to protect the chloroplast during photosynthesis. This poster summarizes some of the observed changes in defined metabolite profiles of plants in response to changes in the activities of carotenoid biosynthetic enzymes.

P 54

TRICHOME CONSTITUENTS OF *CALAMINTHA ASHEI*

Jeffrey D. Weidenhamer-1 and Dale Ray-2
1-Department of Chemistry, Ashland University, Ashland, Ohio 44805 (USA)
2-Department of Chemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-7078 (USA)

The perennial shrub *Calamintha ashei* occurs in the Florida scrub along Florida's Lake Wales ridge. *Calamintha* was previously shown to contain novel, water-soluble menthofuran monoterpenes that inhibit the germination and growth of grasses from the neighboring Florida sandhills. This study was undertaken to characterize the trichomes which dot the surface of *Calamintha* leaves. It was presumed that the trichomes would be found to contain the previously identified menthofurans. However, GC-MS analyses of trichomes individually removed from leaves show no evidence of the menthofurans, but do show three major and several minor volatile constituents that also can be detected in headspace by solid phase microextraction. Mass spectra of these compounds all show a molecular ion at 204, indicative of sesquiterpenes. A bulk extraction of surface compounds was obtained by briefly dipping leaves in dichloromethane, followed by vacuum liquid chromatography and preparative HPLC. The mixture of the three principal constituents has proven extremely difficult to separate, suggesting that the compounds are very closely related isomers. High field (750 MHz) NMR of the mixture was used to elucidate the structure of the major constituent, which is a sesquiterpene with a nine-membered ring. Further work is being undertaken to separate pure samples of each compound by preparative gas chromatographic methods.

P55

The *Arabidopsis brt1* mutant is defective in the gene encoding sinapic acid: UDPG glucosyltransferase

Taksina Sinlapadech, Max O. Ruegger[2], Mike Deak, Clint Chapple[1]
[1]Department of Biochemistry, Purdue University, West Lafayette IN 47907
[2]Dow AgroSciences LLC, 9330 Zionville Road, Indianapolis, IN 46268

Arabidopsis and other members of the Brassicaceae contain sinapate-derived secondary metabolites including a leaf specific ester, sinapoylmalate, a seed specific ester, sinapoylcholine, and their biosynthesic precursor, sinapoylglucose. These compounds are derived from the phenylpropanoid pathway and fluoresce when illuminated under UV light. Arabidopsis mutants blocked in phenylpropanoid biosynthesis have been identified in our lab by their *reduced epidermal fluorescence* (*ref*) phenotype under UV light, a characteristic attributable to decreased levels of sinapoylmalate. Like the *ref* mutants, *brt1* (*bright trichomes 1*) also shares the *ref* phenotype; however, *brt1* plants also exhibit hyperfluorescent trichomes, a phenotype not seen in the *ref* mutants. Mapping of the *brt1* mutation narrowed down the location of the *BRT1* gene to a 37 kb region on chromosome 3 corresponding to the BAC MIL23, which contains 9 predicted coding sequences. One candidate gene in this region encodes an IAA-glucosyltransferase like-protein previously shown to have sinapic acid: UDPG glucosyltransferase activity. Although activity of this enzyme was readily detectable in protein extracts of wild-type seedlings, it was absent in extracts of seedlings homozygous for four *brt1* alleles. Complementation analysis revealed that overlapping genomic DNA fragments containing the gene encoding this glucosyltransferase restored the wild-type phenotype to *brt1* plants. Further, sequencing of 6 *brt1* alleles has identified the mutations responsible for the mutant phenotype. Future experiments to determine how defects in the *BRT1* gene lead to the phenotypes observed in *brt1* plants will focus on a detailed analysis of regulation and localization of the BRT1 protein by using either GUS or GFP fusion protein, analysis of compounds accumulating in *brt1* trichomes, and further characterization of BRT1 enzymatic activity.

P56

Genetic Engineering of Polyketide Biosynthesis in Filamentous Fungi

F. Yu, X. Zhu, R.S. Bojja, L. Du
University of Nebraska, Lincoln, NE.

Filamentous fungi are rich sources for polyketide natural products, including several valuable medicines. These products are synthesized by iterative modular polyketide synthases (PKSs). It remains unclear how the single-moduled PKSs control the highly diverse product structure. The objective of the experiments is to establish a model system for studying the biosynthetic mechanism of fungal polyketides. We have taken a genetic approach to manipulate several PKS genes involved in the biosynthesis of mycotoxins. These PKSs have a similar size and almost identical domain arrangement, yet synthesize polyketides with a chain length varied from 4 to 42 carbons. A gene disruption followed by functional complementation using a heterologous domain/module could yield new products, which may subsequently reveal mechanistic information for the PKSs. Through point-mutation and domain-swapping, we have generated mutants for methyltransferase domain and ketoreductase domain from *Fusarium verticillioides*, the producer of fumonisins. RT-PCR results showed that the heterologous domain was properly transcribed in *F. verticillioides*. HPLC-ELSD and LC-ESMS data demonstrated that the production of fumonisins in these mutants has been eliminated. Experiments are currently progressing toward functional complementation and novel products identification.

P57

REGULATION OF METHYLBENZOATE EMISSION AFTER POLLINATION IN SNAPDRAGON AND PETUNIA FLOWERS

Florence Negre,a Christine M. Kish,a Jennifer Boatright,a Beverly Underwood,b Kenichi Shibuya,b Conrad Wagner,c David G. Clark,b and Natalia Dudarevaa
a Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907. b Department of Environmental Horticulture, University of Florida, Gainesville, FL 32611-0670. c Department of Biochemistry, Vanderbilt University School of Medicine, and Department of Veterans Health Care System, Vanderbilt University, Nashville, Tennessee 37232

Molecular mechanisms responsible for post-pollination changes in floral scent emission were investigated in snapdragon Antirrhinum majus cv. 'Maryland True Pink' and Petunia hybrida cv. 'Mitchell' flowers using a volatile ester, methylbenzoate, one of the major scent compounds emitted by these flowers, as an example. In both species, a 70-75% pollination-induced decrease in methylbenzoate emission begins only after pollen tubes reach the ovary, a process which takes between 35 and 40 h in snapdragon and about 32 h in petunia. This post-pollination decrease in emission is age-dependent and is not triggered by pollen deposition on the stigma. Although petunia and snapdragon both synthesize methylbenzoate from benzoic acid and S-adenosyl-L-methionine (SAM), they use different mechanisms to down-regulate its production after pollination. In petunia, expression of the gene responsible for methylbenzoate synthesis is suppressed by ethylene. In snapdragon, the decrease in methylbenzoate emission is not ethylene dependent. Rather, the combination of a decrease in S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase (BAMT) activity and in the ratio of SAM to S-adenosyl-L-homocysteine (SAH) ("methylation index") after pollination accounts for the decrease in methylbenzoate emission. We found that in both systems scent genes responsible for methylbenzoate synthesis are sensitive to ethylene, however, only petunia uses this plant hormone as a signal for post-pollination down-regulation of methylbenzoate emission.

Index of Authors