30th Annual Mid-Atlantic Plant Molecular Biology Society

Aug. 15th & 16th, 2013

National Wildlife Visitor Center,
Patuxent Research Refuge
Beltsville, MD

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Lots of people provide the support and staffing for this meeting! Many thanks to all of them for the fine job they are doing.

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WELCOME

Welcome to all of you and thanks for coming to the 30th annual Mid Atlantic Plant Molecular Biology Meeting!

It's hard to believe that this is our 30th year, but here we are again to share the stimulation provided by a great palette of speakers in many areas of plant (molecular) biology from around the country. An important goal of this meeting is to help us all keep up-to-date with advances in a broad range of research areas outside our own immediate interests. A second goal is to promote interaction among scientists in a small and informal atmosphere. We therefore provide lunch and breaks at the meeting so each participant has the opportunity to meet invited speakers and presenters.

Our main goal at MAPMBS continues to be: to provide a high-quality, accessible and affordable meeting for scientists in the mid-Atlantic region, and we always welcome your participation, comments and suggestions. Also, if you are interested please join next year's organizing team and volunteer your services in planning next year's MAPMBS meeting. All are welcome at any stage of the planning and organizing process!

Many people are involved in the planning and organizing of this meeting, and we give them our hearty thanks. We wish to especially thank our sponsors, and the exhibitors who furnish us with up-to-date products and services, and help to defray the cost of the meeting.

We thank you for your continued support of and participation in the Mid Atlantic Plant Molecular Biology Society. You can keep up with MAPMBS on our website: http://bioinformatics.towson.edu/mapmbs/default.aspx

Benjamin F. Matthews and Leslie A. Wanner
Co-chairs

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Thursday, August 15, 2013

9:00	Registration and poster set-up		
9:15	Welcome	Ben Matthews and Leslie Wanner	
Session I		**LARGE-SCALE BIOLOGY** Moderator for the morning: Jim Culver, University of Maryland College Park	
9:20	Sheldon McKay *	The iPlant Collaborative: Bringing together high performance computing and biology	Cold Spring Harbor Laboratory
9:50	Ganesh Sriram *	Tools for quantitative dissection of metabolism in plants	Dept. of Chemical and Biomol. Engineering, Univ. of Maryland
10:20		**Coffee , Vendors and Posters**	
10:45	Ian Misner	Novel genome sequences for *Rhizoctonia solani* – Comparative genomics, pathogenicity, and evolution	Computer and Information Sciences, Towson University
11:05	Jorge Vivanco *	Underground communication between roots and the soil microbiome	Center for Rhizosphere Biology, Colorado State University
Session II		**PLANT - MICROBE INTERACTIONS**	
11:35	Boris Vinatzer *	Signatures of natural selection in *Pseudomonas syringae* pv. tomato reveal new insight into plant - microbe interactions	Dept. Plant Pathology, Physiology & Weed Science, Virginia Tech
12:05		Business meeting	
12:20		**LUNCH, Vendors, Posters**	
Session II, cont.		**PLANT - MICROBE INTERACTIONS, cont** Moderator for the afternoon: Alicia Manfre, Hagerstown Community College	
1:40	Arianne Tremblay	Characterization of a new lesion mimic mutant in *Arabidopsis*	Dept. of Biological Sciences, Univ. of Maryland Baltimore County
2:00	Jeanmarie Verchot *	The vital role of cellular chaperones and co-factors as partners in plant-virus interaction	Dept. Entomology and Plant Pathology Oklahoma State University
2:30	Andy Binns *	Host Recognition by *Agrobacterium tumefaciens*	Dept. of Biology, University of Pennsylvania
3:00		**Coffee , Vendors and Posters**	
3:15		Introduction of KEYNOTE SPEAKER: Leslie Wanner, USDA-ARS Beltsville	
3:20	**Gregg Howe ***	**Induced resistance to herbivores**	MSU-DOE Plant Research Laboratory, Michigan State University
4:30	close of day (building closes)		

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Friday, August 16, 2013

9:00	Registration , Vendors and Posters		
Session III		**Genetics and Genomes** Moderator for the morning: David Puthoff, Frostburg State University	
9:15	Tim McNellis	Genes with trait-associated expression patterns in an F1 population exhibit physical grouping on the apple (*Malus domestica*) genome	Plant Pathology & Environmental Microbiology Dept. Pennsylvania State University
9:35	J. Antoni Rafalski *	Genetic and epigenetic diversity of maize: A plant breeding perspective	DuPont Pioneer, Genetic Discovery Group, Wilmington, DE
10:05		**Coffee , Vendors and Posters**	
11:00	John Carlson *	Genomics of stress responses in trees	Dept. of Ecosystem Science and Management, Pennsylvania State University
11:30	Ann Loraine *	Alternative splicing under stress – Noise or adaptation	Dept. of Bioinformatics and Genomics, University of North Carolina at Charlotte
12:00		**Lunch , Vendors and Posters**	
Session III, cont.		**Genetics and Genomes** Moderator for the afternoon: Donna A. Lalli, Ph.D. Biotechnologist USDA/APHIS/BRS	
1:15	Fow-Sen Choa	Cell electricity from dissected plants and its responses to radiation and cascading	Dept. of CSEE, University of Maryland Baltimore County, Baltimore
1:35		Introduction of KEYNOTE SPEAKER: Ben Matthews	
1:40	**Pam Green ***	**Genome-wide analysis of miRNAs, target RNAs, and mRNA decay**	Delaware Biotechnology Institute and University of Delaware
2:40	Lev Nemchinov	Using transcriptome resources for identification of long non-coding RNAs in *Medicago sativa*	USDA-ARS, Molecular Plant Pathology Laboratory
3:00	**Close of day.**	**Thanks for your participation!**	**Please take down your posters**

* Invited speaker

Posters – Titles, Authors, and Afilliations (Please view posters during lunch, breaks, etc)

Poster #	Abstract is on page:	Title, Authors, Affiliations
1	21	**INTERACTIONS OF CYCLIC DROUGHT, CARBON DIOXIDE ENRICHMENT AND DIURNAL FACTORS ON METABOLITES IN POTATO LEAFLETS** Jinyoung Y. Barnaby[1], David Fleisher[1], Vangimalla Reddy[1] and Richard Sicher[1] [1]USDA-ARS, Crop Systems and Global Change Laboratory, Room 342, Building 001, BARC-west, 10300 Baltimore Avenue, Beltsville, Maryland, 20705 USA Email of corresponding author: Jinyoung.barnaby@ars.usda.gov
2	21	**TOBACCO MOSAIC VIRUS TARGETS HOST TRANSCRIPTIONAL REGULATORS EXPRESSED IN THE VASCULAR TISSUE** Tamara D. Collum and James N. Culver University of Maryland College Park 5115 Plant Sciences Bldg, College Park, MD Email of corresponding author: tdfisch@umd.edu
3	22	**A SYNTHETIC *TOBACCO MOSAIC VIRUS*** Bret Cooper and Kimberly Campbell Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD Email of corresponding author: bret.cooper@ars.usda.gov
4	22	**SUPERIOR CROSS-SPECIES REFERENCE GENES -BLUEBERRY GENE EXPRESSION STUDIES-** Jose V. Die, Lisa J. Rowland US Department of Agriculture, Agricultural Research Service, Genetic Improvement of Fruits and Vegetables Laboratory, 10300 Baltimore Avenue, BARC-West, Beltsville, MD 20705 Email of corresponding author: Jose.Die@ars.usda.gov Twitter: @jdieramon
5	23	**LRS1, WDR20, AND UBP3 FUNCTION IN A DEUBIQUITINASE COMPLEX IN ARABIDOPSIS** Les Erickson[1], Elitsa Ananieva[2], Janet Donahue[2] and Glenda Gillaspy[2] 1. Dept. of Biological Sciences, Salisbury University, Salisbury, MD 2. Dept. of Biochemistry, Virginia Tech, Blacksburg, VA Email of corresponding author: flerickson@salisbury.edu
6	24	**ANALYSIS OF THE SOYBEAN *(Glycine max)* ROOT *CIS*-REGULOME UPON TREATMENT WITH VARIOUS PHYTOHORMONES** Parsa Hosseini[1,2], Reham Youssef[2], and Benjamin F. Matthews[2] [1]George Mason University, School of Systems Biology, Manassas, VA. [2]USDA-ARS Soybean Genomics and Improvement Laboratory, PSI
7	24	**EFFECTS OF ELEVATED TEMPERATURE ON CAROTENOID BIOSYNTHESIS IN THE REPRODUCTIVE ORGANS OF THE DIPLOID STRAWBERRY, *FRAGARIA VESCA*** Melantha Jackson[1,2], Frederick Khacik[2], and Janet P. Slovin[1] [1]USDA/ARS Genetic Improvement of Fruits and Vegetables Lab, Beltsville, MD 20705 [2]Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742

14	**30**	**INVESTIGATING THE FUNCTION OF THE N-TERMINAL DOMAIN OF EIN2, AN NRAMP HOMOLOG, IN ETHYLENE SIGNALING IN ARABIDOPSIS** Jennifer M. Shemansky[a], Mathieu Cellier[b], Caren Chang[a] (a) Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742 (b) INRS – Institut Armand-Frappier Research Centre, Laval (Quebec) Canada. Email of corresponding author: carenc@umd.edu
15	**30**	**ECTOPIC EXPRESSION OF *AtPAD4* BROADENS RESISTANCE OF SOYBEAN TO SOYBEAN CYST AND ROOT-KNOT NEMATODES** Reham M. Youssef[1,3], Margret H. MacDonald[1], Eric P. Brewer[1], Gary R. Bauchan[1], Kyung-Hwan Kim[2], Benjamin F. Matthews[1*] [1]USDA-ARS, Soybean Genomic and Improvement Laboratory, 10300 Baltimore Ave, Beltsville,MD 20705. [2]Cell and Genetics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-100, South Korea. [3] Plant Protection Departments, Faculty of Agriculture, Fayoum University, Fayoum, Egypt Email of corresponding author: Ben.Matthews@ars.usda.gov
16	**31**	**ARABIDOPSIS TSO1 REGULATES MALE FLORAL ORGAN DEVELOPMENT AND MICROGAMETOGENESIS** Julie Caruana, Wanpeng Wang, Paja Sijacic, Charles Hawkins, and Zhongchi Liu Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD corresponding author: zliu@umd.edu
17	**32**	**DETECTION AND IDENTIFICATION OF PLANT VIRUSES BY MICROARRAY** David C. Henderson[1], Basavaraj Bagewadi[2], Kael Fischer[3], Ramon L. Jordan[1], David Wang[4], Keith L. Perry[5], Ulrich Melcher[6], Claude Fauquet[2], John Hammond[1] [1]USDA-ARS, Floral and Nursery Plants Research Unit, USNA; [2]Danforth Plant Science Center, ILTAB, St. Louis, MO; [3]University of Utah, Department of Pathology, Salt Lake City, Utah; [4]Washington University, Departments of Molecular Microbiology and Pathology & Immunology, St. Louis, MO; [5]Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Ithaca, NY; [6]Oklahoma State University, Department of Biochemistry and Molecular Biology, Stillwater, OK david.henderson@ars.usda.gov

Speaker Abstracts

THE iPLANT COLLABORATIVE: BRINGING TOGETHER HIGH PERFORMANCE COMPUTING AND BIOLOGY

Sheldon McKay, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
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The *iPlant* Collaborative (www.iplantcollaborative.org) is an NSF-funded virtual center charged with the development cyberinfrastructure to support a broad range of life science research. iPlant offers access to resources for high performance computing, data storage, and analytical software needed for a variety of data- or compute-intensive research applications. This presentation will give an overview of major components of iPlant's CI, including the Discovery Environment, the Data Store, the Foundation API (advanced programming interface) and the DNA Subway, and highlight selected collaborations with the research community. The Discovery Environment is a powerful web portal for managing data, conducting analyses, and building analytical workflows. Complex bioinformatics applications can be run without the command line, and users can customize the platform by integrating their own software tools. The Data Store offers secure and scalable, cloud based data storage. iPlant's Atmosphere offers free cloud computing analogous to Amazons EC2 (Elastic Compute Cloud) platform. The Foundation API provides web services for developing applications that can access high performance compute resources in the XCEDE (Extreme Science and Engineering Discovery Environment) supercomputer network. The DNA Subway is a web-based application that offers an intuitive user interface for research and education using a number of analytical workflows, including genome annotation, DNA barcoding, phylogenetics and RNA-Seq.

TOOLS FOR QUANTITATIVE DISSECTION OF METABOLISM IN PLANTS

Ganesh Sriram (presenter and corresponding author), Shilpa Nargund, Ashish Misra, Xiaofeng Zhang, Gary D. Coleman

Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, 1208D Bldg. 090, College Park, MD 20742
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Investigation of metabolism in plant cells is crucial to understanding how they respond to their environment. Isotope labeling experiments and metabolic flux analysis enable a quantitative dissection of carbon traffic in metabolism. Here, we present our recent work on elucidation of metabolic flux in Arabidopsis and poplar cell suspensions. This presentation will specifically discuss the development of computational and experimental tools to study these interactions. First, we computationally designed isotope labeling experiments that would facilitate precise evaluation of fluxes in pathways of interest. Next, we performed parallel labeling experiments utilizing a number of isotopically labeled forms of glucose on the cell suspensions. An important result originating in these experiments is that significant recycling of initially present carbon can occur during the labeling experiments, and this "reflux" needs to be factored into the flux evaluation to render it accurate. Subsequent

labeling experiments and measurements in situations that did and did not favor recycling provided information toward accurate flux maps. Finally, we will discuss correlations between flux maps and gene expression measurements.

NOVEL GENOME SEQUENCES FOR *RHIZOCTONIA SOLANI* – COMPARATIVE GENOMICS, PATHOGENICTY, AND EVOLUTION.

I. Misner (1), O. Darwish (1), M. Nair (3), D.P. Roberts (2), N. Alkharouf (1), A. Pain (3), and D.K. Lakshman (2)

(1) Computer and Information Sciences, Towson University, Towson, MD, USA; (2) Floral & Nursery Plants Research Unit and Sustainable Agricultural Systems Laboratory, USDA-ARS, Beltsville, MD, USA; (3) Computational bioscience Research Center, King Abdullah University of Science and Technology, Thuwal, KSA.

The Basidiomycete fungus *Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*) is a species complex spanning over 100 members. These soilborne fungi cause blights, wilts, and damping off of more than 188 plant species covering major cultivated plants. Members of *R. solani* are divided into 14 anastomsis groups (AG), some of which are further divided into intraspecific groups. We have utilized next-generation sequencing and comparative genomics to generate six draft genomes from members of multiple AGs spanning diverse host ranges and pathologies. The genomes range in size from 37-60 MB and show a wide variety of intraspecific variations including gene copy number, intron diversity, and synteny. Gene content was generated for each of the six draft genomes, along with four additional isolates, using RNAseq and bioinformatic tools. We compared genes across 10 isolates, in order to identify gene families, genes involved in pathogenesis, and gene family duplication/loss events. This study represents the most complete analyses of members of *R. solani* to date and highlights key events in the evolution and pathogenic nature of this important plant pathogenic fungus.

PLANT HEALTH AND THE SOIL MICROBIOME

Jorge M. Vivanco

Professor of Horticulture; Colorado State University

The underground world is a lively place, with plant roots, soil microbes, and other underground organisms waging a continual battle for resources. Plant roots take an active role in this conflict through the exudation of various chemicals, yet key areas of this process remain mysterious: what genes and gene networks control exudation? How do plant root exudates influence the microbial community of the soil? Finally, if plant roots do play a large role in soil microbial composition, what effect does plant biodiversity and agricultural management have on soil microbial diversity?
Bringing together a combined understanding of root-microbiome interactions is likely to redefine the goals and practices of agricultural management and sustainability. These and other ideas will be discussed in the presentation.

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Recent references related to the presentation:
1. Sugiyama A, Bakker MG, Badri DV, Manter DK, Vivanco JM (2013) Relationships between Arabidopsis genotype-specific biomass accumulation and associated soil microbial communities. Botany 19:123-126
2. Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013). Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytologist 198:264-273
3. Carvalhais LC, Dennis PG, Kidd BN, Badri DV, Tyson GW, Vivanco JM, Schenk PM (2013) Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. PLoS ONE 8(2): e56457. doi:10.1371/journal.pone.0056457
4. Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013). Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic related compounds predominantly modulate the soil microbiome. Journal of Biological Chemistry 288:4502-4512
5. Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS ONE 8(2): e55731. doi:10.1371/journal.pone.0055731
6. Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant and Soil 360:1-13 (*Marschner Review*)
7. Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012). Manipulating the soil microbiome to increase plant health and soil fertility. Biol Fertil Soils 48:489-499

SIGNATURES OF NATURAL SELECTION IN *PSEUDOMONAS SYRINGAE* PV. *TOMATO* REVEAL NEW INSIGHT INTO PLANT - MICROBE INTERACTIONS

Christopher R. Clarke, Boris A. Vinatzer

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Pathogens are under continuous selection pressure to evade or interfere with their recognition by the immune system of their hosts while hosts are under continuous selection pressure to maintain or improve recognition of invading pathogens. In animals and plants the first line of defense consists in immunity triggered by pathogen-associated molecular patterns (PAMPs). Flagellin is the archetypical PAMP and is widely recognized by animals and by plants. A 22 amino acid long epitope of flagellin has been intensively studied for many years and its plant receptor Fls2 is well known. Comparing almost identical strains of the tomato pathogen *Pseudomonas syringae* pv. *tomato* we identified a second region of flagellin, called flgII, to be under selection for avoidance of recognition by the tomato immune system. We now found that flgII triggers immunity in many other Solanaceous species. Interestingly, different alleles of flgII are differentially recognized by different plant species suggesting that *P. syringae* adapts to specific hosts (or groups of hosts) through allelic variation. This minimizes the immune response triggered in the host but increases the immune response triggered in some non-hosts. To investigate the mechanism of flgII recognition we tested if Fls2 is the flgII receptor. Silencing *FLS2* in tomato and expressing the *FLS2* gene of tomato in *Nicotiana benthamiana* we determined the flgII recognition is not *FLS2*-dependent. To start identifying the flgII receptor in tomato, we surveyed many different tomato accessions and

found two accessions that do not respond to flgII. We now made crosses between these flgII-insensitive accessions and flgII-sensitive tomato cultivars to map and clone the flgII receptor.

CHARACTERIZATION OF A NEW LESION MIMIC MUTANT IN ARABIDOPSIS

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Successful control of plant diseases relies on a thorough understanding of the complex defense gene networks governing plant responses to pathogen invasion. It is critical to identify defense related genes, understand their function, and delineate their interactions with other defense genes. Characterization of an array of lesion mimic mutants has led to the discoveries of several novel defense-related genes that regulate plant innate immunity. Here we report the characterization of a new lesion mimic mutant, designated *lmm100*, in the background of another mutant ordered from the ABRC seed stock center. *LMM100* was cloned via map-based cloning and sequencing revealed that *LMM100* encodes a protein involved in mitochondria function. The cloning of *LMM100* was validated by complementation of the mutant with a wt *LMM100* gene. The *lmm100* mutant shows cell death in the leaves up to the sixth-leaf stage, starting from the middle vein of a leaf and progresses more severely as the leaf becomes older. Protoplasts of *lmm100* are more sensitive to a chemical that induces cell death, compared with wild-type protoplasts. The *lmm100* mutant shows enhanced resistance to *Pseudomonas syringae* and accumulates higher SA levels than wild-type, in an EDS1-dependent manner. We are in the process of further characterizing the function of the *LMM100* gene in regulating programmed cell death and plant innate immunity.

THE VITAL ROLE OF CELLULAR CHAPERONES AND CO-FACTORS AS PARTNERS IN PLANT VIRUS INFECTION

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Cellular chaperones and folding enzymes play central roles in RNA virus infection. Most cellular chaperones contribute to protein quality control in the cell, but with regard to virus infections, they also contribute to the formation of viral multi-protein complexes. In recent years many labs have examined the central role of key cellular chaperones in promoting successful formation of viral protein complexes and regulating the cellular environment to promote infection. Certain examples suggest that chaperones are diverted from cellular functions and employed by viruses to support replication. There are other examples that suggest plant viruses piggyback onto the established cellular systems without dismantling pathways to promote infection. For example, HSp70, Hsp90, Yjd1, and Hsp40 play central

roles in the formation of membrane bound replication complexes for many RNA viruses and are also vital cellular factors in the cell driving substrate protein folding and disaggregation. Hsp70 family of proteins can interact with a wide range of cofactors and folding substrates and contribute to diverse biological processes. The most common cofactors are J-domain proteins (also known as Hsp40) which identify and recruit substrates to Hsp70 through direct interactions. HSP90 is also known to cooperate in the maturation of certain client proteins. There are several co-chaperones, that associate viral processes involved in intercellular transport. For example, DNAJ proteins contribute to membrane bound events relating to virus intercellular movement. For example, RME-8, interacts with the pomovirus TGB2 movement protein . RME-8 localizes to endocytic vesicles and interacts with cytosolic Hsp70 to control clathrin-dependent endocytosis. Plant chaperone systems in the endoplasmic reticulum (ER) are reported to support virus cell-to-cell movement. For example, TMV relies on calreticulin residing in the plasmodesmata to enable cell-to-cell movement. The potexvirus TGB3 protein stimulates expression of ER resident chaperones via the bZIP60 transcription factor. Up-regulating factors involved in protein folding may be essential to handling the load of viral proteins translated along the ER. Understanding how plant viruses interact with the cell's chaperone machinery is critical to developing new strategies to control disease and for identifying cellular targets for engineering anti-viral control measures.

HOST RECOGNITION BY *AGROBACTERIUM TUMEFACIENS*

Andrew N. Binns

Department of Biology, University of Pennsylvania

Agrobacterium tumefaciens is a broad host range plant pathogen that resides, as a saprophyte, in soil utilizing decomposing organic matter as nutrition. However, when virulent forms of the bacterium find themselves in close contact with plants – particularly angiosperms – they undergo an extensive alteration in their physiology enabling transfer of DNA (the T-DNA) from their "tumor inducing" (Ti) plasmid into the plant cell and its integration into the plant chromosomal DNA, ultimately resulting in tumorigenesis. This capacity for DNA transfer is, of course, the basis for construction of a wide variety of transgenic plants. The resources required of the bacterium to carry out the pathogenic steps are considerable and for this reason, the 'switch' to activate the metabolic pathways should (and does) occur only in the presence of plants. *A. tumefaciens* utilizes a two component system, VirA (histidine kinase) and VirG (response regulator), as well as the periplasmic sugar binding protein, ChvE, to control expression of the virulence (*vir*) genes that mediate T-DNA transfer. Together, VirA and ChvE are capable of recognizing phenolic derivatives, a variety of monosaccharides and low pH (5.0-5.5). When all three of these signals are present in appropriate concentrations, VirA phosphorylates VirG, which subsequently induces *vir* gene expression. Here I will present work done on the following question - *How does* A. tumefaciens *identify diverse host chemical signals in order to achieve a broad host range?* The ChvE/VirA signal perceiving system is a model for understanding how two component systems can combine signal diversity with tight control of gene expression. I will focus on experiments that illuminate the physical and cellular processes that affect the capacity of ChvE to serve as the sugar sensor, and how this affects the natural host range of Agrobacterium.

Thursday's Keynote Address

INDUCED RESISTANCE TO INSECT HERBIVORES

Gregg A. Howe

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Plants and insect herbivores are engaged in a co-evolutionary battle to eat or not be eaten. Interactions between these two groups of organisms—which comprise the most species-rich multicellular lineages on earth—have dramatically shaped organismic diversity in terrestrial ecosystems. Here, I will review our efforts to elucidate the molecular mechanism by which the plant hormone jasmonate (JA) controls induced defense responses to insect herbivores. Areas of progress includes the identification of the bioactive form of JA, identification of JAZ repressors as the substrates for the E3 ubiquitin ligase SCF^{COI1}, and discovery that COI1-JAZ complexes function as receptors for JA. We recently delineated two mechanisms to arrest JA signaling, including production of JAZ alternative splice variants that fail to assemble coreceptor complexes, and cytochrome P450-mediated catabolism of JA. Recent progress in understanding how a simple hormone controls myriad defense responses will be presented.

GENES WITH TRAIT-ASSOCIATED EXPRESSION PATTERNS IN AN F_1 POPULATION EXHIBIT PHYSICAL GROUPING ON THE APPLE (*MALUS* × *DOMESTICA*) GENOME

Philip J. Jensen[1], Gennaro Fazio[2], Naomi Altman[3], and Timothy W. McNellis[1,4]

[1]Plant Pathology & Environmental Microbiology Dept., 211 Buckhout Laboratory, Penn State Univ., University Park, PA, 16802; [2]Plant Genetics Resources Unit, USDA, ARS, 630 W. North St., Geneva, NY, 14456; [3]Department of Statistics, 323 Thomas Building, Penn State Univ., University Park, PA, 16802; [4]Corresponding author
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We applied DNA microarray transcription profiling to 48 individual F_1 apple (*Malus* × *domestica*) trees from a cross of two highly heterozygous parents, aiming to identify gene expression markers (GEMs) whose transcript abundance in actively-growing shoot tips was associated with specific traits of agricultural interest (trait-associated GEMs, or TAGEMs). By dividing the trees into phenotypic groups and using standard differential expression analysis, we found that TAGEMs often physically clustered in the vicinity of the trait locus. For example, of 26,000 unique transcripts queried, 30 transcripts whose abundance was associated with the powdery mildew (*Podosphaera leucotricha*) disease resistance phenotype (q-value < 0.05) were identified, and the physical locations of the genes encoding these transcripts on the ~742 Megabase (Mb), 17-chromosome apple genome were determined. Twenty-three of the genes were located on chromosome 12, with 19 of these clustering within 5 Mb of the major powdery mildew quantitative trait locus in the population. Similar results were obtained for woolly apple aphid resistance and several molecular traits. This phenomenon has potential to rapidly identify linked molecular markers.

GENETIC AND EPIGENETIC DIVERSITY OF MAIZE: A PLANT BREEDING PERSPECTIVE

J. Antoni Rafalski

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Recent rapid technological developments, such as high density SNP genotyping, reduced genome representation sequencing, comparative genomic hybridization, whole genome sequencing and, more recently, epigenetic inheritance analysis expanded the scope of tools useful for probing genetic diversity of individuals and populations. Using these tools we and others were able to describe in detail the complex genomic structure and genetic diversity of cultivated maize and compare it to ancestral populations. I will show how we use this information in connection with field-determined phenotypes and statistical tools, such as candidate gene and whole genome association mapping (GWAS), as well as positional cloning, to develop genetic diagnostic assays for maize breeders and to improve the understanding of hybrid vigor.

GENOMICS OF STRESS RESPONSE IN TREES

John E. Carlson

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The sustainability of both natural and managed ecosystems is increasingly threatened by exotic pests, diseases, invasive plants, and climate change. However, genomic resources and genetic tools specific to biotic and abiotic stresses are not available for many important species, such as forest trees. We are producing expressed gene sequence databases for studying environmental stresses such as drought, heat, cold, air pollution, insects and disease for a phylogenetically wide distribution of hardwood (Angiosperm) forest tree species including *Acer saccharum*, *Fagus grandifolia, Fraxinus pennsylvanica, Gleditsia triacanthos*, *Juglans nigra*, *Liquidambar styraciflua*, *Liriodendron tulipifera*, *Nyssa sylvatica*, *Populus, Prunus serotina,* and *Quercus rubra*. In addition, we have a whole genome sequencing project underway for Chinese chestnut (*Castanea mollissima*) targeting response to the chestnut blight disease. These genomic resources are being used for differential gene expression, gene network, and comparative genomics studies to gain a better understanding of the genetic basis of responses to environmental stresses in forest trees.

ALTERNATIVE SPLICING UNDER STRESS – NOISE OR ADAPTATION?

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Most genes in higher eukaryotes contain introns, regions that are transcribed into RNA but require removal before the RNA can be translated into protein. The process of removing intronic sequence from RNA, called splicing, involves assembly of the splicing machinery onto the newly synthesized RNA, recognition and excision of introns, and ligation (splicing) of flanking sequences called exons. For many genes, this splicing reaction can happen in different ways, leading to production of RNA isoforms that retain different sequence segments that in some cases change the function of the encoded protein. This phenomenon, called alternative splicing, is thought to affect the majority of protein-coding, multi-exon plant genes. How the splicing machinery determines which sequences should be removed or retained is not well understood, but for many years it has been known that a subset of genes involved in splicing are differentially expressed under heat, cold, and water deficit stresses, suggesting that plant cells possess a mechanism to maintain splicing fidelity in the face of daily or seasonal fluctuations in temperature and water availability. Thanks to development of new sequencing technologies, especially ultra-high throughput sequencing of cDNA (RNA-Seq), it is now possible to survey splicing in different sample types on a genome-wide scale. In this talk, I'll discuss analysis of data from RNA-Seq experiments from diverse sample types, focusing on an experiment in which we subjected *Arabidopsis* plants to a severe water deprivation stress. Consistent with prior studies, we observed many genes to be

differentially expressed under the treatment, but we also observed changes in splicing patterns, the most significant of which affected genes involved in splicing.

CELL ELECTRICITY FROM DISSECTED PLANTS AND ITS RESPONSES TO RADIATION AND CASCADING

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The phenomenon of electrical potential differences along the plant apoplast has been reported for more than a century. Both action potential (AP) and variation potential (VP) are utilized by plants to increase the speed of communication and monitor information about its own environment and health. In this work, we show that if we dissect a segment from a tree branch, about the same amount terminal voltage was still measured on each one of the two separated sections. The amount of voltage didn't seem to be reduced. If we further subdivide each piece, each new and smaller piece was able to reproduce the nearly same amount of terminal voltage. To understand the source of this bio-electricity, we cut a slice of pine trunk as shown in Fig. 1 (b). We pasted one side of the disk with silver epoxy and covered it with alumina foil to form an electrode. We then measure the generated short circuit current with a silver coated probe and a pico-ampere meter. We found that the maximum current was coming from close to the vascular cambium belt area as shown in Fig. 1 (a). The measured short circuit current is mapped along the radius from the center to the perimeter of the circle. From the current plot it seems that the source of the bio-electricity is generated from living cells close to the vascular cambium and secondary xylem and phloem areas. The two sides of a dissected tree stick can be pasted with silver epoxy and alumina foil to form a small living battery cell as shown in Fig. 1(c). We have cascaded multiple dissected tree sticks in serial and parallel connections and show stable combined current up to 40μA with 16 parallel sticks and up to 800mV voltage with 3-4 serially cascaded stages. The source of the polarity may be related to directional water transport protein like acquaporin in plant cells. Details are under studies. We also observed immediate and constant terminal voltage drops on both a full tree and a dissected tree segment cases when they are illuminated with milli-rem range intensity of radioactive sources like neutron, x-ray, gamma, and beta rays. When radiation sources were removed, recovery of terminal voltage will also immediately take place. The recovery time is different for different radiation sources. Details are still under studies.

Friday's Keynote Address

GENOME-WIDE ANALYSIS OF miRNAs, TARGET RNAs, AND mRNA DECAY

Pam Green[1-4], Dong-Hoon Jeong[1,2], Shawn Thatcher[1,3,5], Vinay Nagarajan[1,2], Rebecca Brown[1,4,6], Sunhee Park[1,2], Jixian Zhai[1,2], Sai Guna Ranjan Gurazada[1,5], Emanuele De Paoli[1,7], Linda Rymarquis[1,8], and Blake Meyers[1,2]

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MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression that control development, stress responses and other processes. They most often function by guiding cleavage of target mRNAs. The resulting cleavage products are subsequently degraded by cellular RNA-degrading enzymes including XRN4. Although deep sequencing of miRNAs has been routine for several years, new miRNAs and new regulation of known miRNAs are still being discovered even from the most well-studied plants. Examples from large studies we carried out in Arabidopsis and rice include cases of miRNA regulation in response to cold, heat, drought, submergence, metal stress, and many examples of tissue or organ preferential expression (Jeong et.al., Plant Cell, 2011, 23:4185-207; Jeong et al., Plant Phys., 2013, 162:1225-45). Among the new cases of miRNA regulation were distinct miRNA family members exhibiting differential accumulation in different organs that impact target selection and cleavage. In rice, our findings related to miR529 are particularly intriguing because they add new insight about how an agriculturally significant phenotype may be controlled. The analysis of miRNAs was enhanced by Parallel Analysis of RNA Ends (PARE), an approach developed to sequence the cleaved targets of miRNAs genome-wide. PARE data was most powerful when interpreted with the emerging diversity of miRNA variants, ARGONAUTE immunoprecipitation data, and small RNA expression data in Arabidopsis. This led to a better understanding of miRNA regulation, cleavage function, and insights about tissue-specific expression and pathogen responses that would not have been possible without this combinatorial approach. An underdeveloped application of PARE is its use for analysis of the RNA degradome, that is the entire population of partially degraded mRNA molecules, of which cleaved miRNA targets represent a small proportion. To better understand the role of XRN4 in general RNA decay, we examined PARE and RNA-Seq data from polyA+ and polyA- libraries made from wild-type and *xrn4* mutant plants. Our results indicate that transcripts affected by XRN4 are degraded by multiple pathways and emphasize the role of XRN4 as a major player in mRNA turnover in Arabidopsis. Funded by the NSF, DOE and USDA.

USING TRANSCRIPTOME RESOURCES FOR IDENTIFICATION OF LONG NON-CODING RNAs IN *MEDICAGO SATIVA*

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Long non-coding RNA (lncRNAs) are larger than all types of small regulatory RNAs and are usually comprised of at least 200 nts. The pool of data suggesting involvement of lncRNAs in diverse cellular functions is growing exponentially. While there are many lncRNA-related studies on mammalian transcriptomes, only a small amount of data is available on lncRNAs in plants. Our interests lie in identification and understanding regulatory roles of lncRNAs in alfalfa (*Medicago sativa*), the most extensively cultivated forage legume in the world. According to a comprehensive ncRNA sequence database fRNAdb, only eight ncRNAs have previously been identified in alfalfa. We have expanded the list of alfalfa lncRNAs and explored their functional characteristics by computational analysis and by profiling expression of lncRNAs in response to salt stress. Using a computer program, Coding Potential Calculator (CPC), which discriminates coding from non-coding transcripts, and a standalone blastx to confirm CPC output, we were able to predict 25,662 non-coding sequences in alfalfa root transcriptome out of 89,310 initial transcripts that were entered into CPC. Only 93 transcripts shared a high degree of similarity (83-100%) to previously annotated plant ncRNAs. A majority of these sequences serve as precursors for small RNAs, and only a few are possibly functional as long molecules. Several of the latter, non-precursor transcripts, had blast hits with ENOD40 family, which is involved in root nodule organogenesis and may act as a dual RNA with characteristics of both protein coding and non-coding RNAs. Other lncRNAs included tentative Group II introns, members of a large class of catalytic RNAs and retrotransposable elements and GUT15 transcripts, unstable ncRNAs that may have regulatory functions in plants. The remaining 25,585 unclassified sequences were scanned against genic (coding), intragenic (introns), and intergenic regions of the genome of *M. truncatula* using blastn tool. This resulted in identification of 10,901 sequences (12.2% of the starting transcripts) that had blast hits with non-coding regions of *M. truncatula*. Further elucidation of true lncRNAs from this pool of candidates is currently being conducted by using different prediction algorithms and by analyzing transcripts' ability to form secondary structures. Apart from identification of lncRNAs in alfalfa and understanding their functional roles in developmental and stress adaptation pathways, we are also interested in transcriptional profiling of lncRNAs in order to find potential biomarker candidates among differentially expressed transcripts (DETs). Examination of all putative lncRNAs revealed 614 DETs in alfalfa under salt treatment. The amount of DETs in the salt-tolerant line exceeded that in the salt-sensitive line by more than two-fold. This may point to the engagement of lncRNAs in regulation of salt-responsive genes.

Poster abstracts

Poster #1

INTERACTIONS OF CYCLIC DROUGHT, CARBON DIOXIDE ENRICHMENT AND DIURNAL FACTORS ON METABOLITES IN POTATO LEAFLETS

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Drought risk is exacerbated by climate change. Loss of food production as a result of climate change and an increasing world population, particularly future expected drought frequency and intensity is a major challenge. Plant responses to water stress have been well studied; however, plant responses to cyclic droughts combined with other environmental factors, such as CO_2 enrichment and diurnal light/dark cycle have not been examined in detail. In this study, cyclic drought was imposed at post-tuber initiation (R) or at both vegetative and post-tuber initiation (VR) growth stages of potato (*Solanum tuberosum* L. cv Kennebec). Experiments were conducted in outdoor naturally sunlit soil-plant-atmosphere research chambers located in Beltsville, MD. We showed that pre-drought treatment improved water stress tolerance during a subsequent drought cycle, except when plants were severely stressed. The drought tolerance was enhanced when plants were treated under elevated CO_2 condition. The degree of water stress, i.e., none, mild (~ -1.0 MPa), or severe stress level (~ -1.4 MPa), was the dominant factor in clustering metabolites with similar responses to drought and this was followed by the effect of pre-drought treatment. Diurnal effects (morning or afternoon) were visible under mild or severe water stress and under both ambient and elevated CO_2.

Poster #2

TOBACCO MOSAIC VIRUS TARGETS HOST TRANSCRIPTIONAL REGULATORS EXPRESSED IN THE VASCULAR TISSUE

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The tobacco mosaic virus (TMV) replication protein can interact with select auxin transcriptional regulators, IAA26, IAA27 and IAA18. Previously we reported that IAA26 is expressed in the phloem vascular tissue, leading us to hypothesize this interaction may be advantageous for the systemic movement of the virus. We have now determined the expression patterns of IAA27 and IAA18 using a GUS reporter assay. While all three interacting IAAs are expressed in the vascular tissue, they each have a unique expression pattern. IAA26 is observed in the veins throughout the leaf, IAA27 is expressed only in the major vein, and IAA18 is expressed in the veins located at the distil region of the leaf. IAA26 and IAA27 expression is also observed in roots. We next infected IAA26 knock-out plants

with TMV to determine the effect the absence of IAA26 has on virus accumulation in inoculated and systemic tissue. We inoculated both young (4-week) and old (8-week) plants. Leaf punches were collected from inoculated and systemic leaves. Accumulation of virus was determined by western blot analysis and quantified using CP standards. In young plants accumulation of virus is similar in wild-type and IAA26 knock-out lines. While in older plant TMV accumulates to lower levels in IAA26 knock-out plants compared to wild-type. Furthermore a mutated form of the virus, TMV-V1087I, which cannot interact with IAA26, displays attenuated disease symptoms and a reduced ability to travel systemically in older plants. We hypothesize that by interacting with IAAs TMV alters gene regulation within the vascular tissue and this interaction is of particular importance in older tissues.

Poster #3

A SYNTHETIC *TOBACCO MOSAIC VIRUS*

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A linear, non-self-replicating, DNA template of *Tobacco mosaic virus* (TMV) was enzymatically synthesized *in vitro* from 120 overlapping oligonucleotides designed from the alphabetic text rendering of the first TMV genome sequence which was elucidated by Goelet *et al.* (1982). RNA was transcribed from the template *in vitro*, encapsidated with purified TMV capsid protein (CP) *in vitro,* and inoculated to tobacco plants. The plants did not develop symptoms. When two nucleotide mutations present in the Goelet sequence, but not present in most other TMV sequences in Genbank, were altered to reflect the consensus, the derivative synthetic virions produced classic TMV symptoms on tobacco. A chimera DNA template was then created by substituting the TMV CP gene with oligonucleotides for the homologous *Tomato mosaic virus* (ToMV) CP gene. Virions from the chimera elicited hypersensitivity on *Nicotiana sylvestris*, normally a systemic host for wild-type TMV. This is the first report of the chemical synthesis of an infectious plant virus nucleic acid. The method is useful for confirming genome sequencing results and for easily engineering chimera viruses in the absence of biological progenitor material.

Poster #4

SUPERIOR CROSS-SPECIES REFERENCE GENES -BLUEBERRY GENE EXPRESSION STUDIES-

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http://bioinformatics.towson.edu/mapmbs/default.aspx

The advent of affordable Next Generation Sequencing technologies has had major impact on studies of many crop species, in which access to genomic technologies and genome-scale data sets have been extremely limited until now. The recent development of genomic resources in blueberry will enable the application of high throughput gene expression approaches that should relatively quickly increase our understanding of blueberry physiology. These studies, however, require a highly accurate and robust workflow and make necessary the identification of reference genes with high expression stability for correct target gene normalization. To create a set of superior reference genes for blueberry expression analyses, we mined a publicly available transcriptome data set from blueberry for orthologs to a set of *Arabidopsis* genes that showed the most stable expression in a developmental series. In total, the expression stability of 13 putative reference genes was evaluated by qPCR and a set of new references with high stability values across a developmental series in leaves, fruits and floral buds of blueberry were identified. We also demonstrated the need to use at least two, preferably three, reference genes to avoid inconsistencies in results, even when superior reference genes are used. The normalization strategy presented here is a prerequisite for functional genomics and our study provides ample possible reference genes for use in blueberry experimental research based on transcriptome data mining and experimental validation. This work is described in light of the current standards of qPCR and the development of new approaches seeking to cultivate better practices in quantitative experiments. The new references identified here will provide a valuable resource for accurate normalization of gene expression in *Vaccinium* spp. and may be useful for other members of the Ericaceae family as well.

Poster #5

LRS1, WDR20, AND UBP3 FUNCTION IN A DEUBIQUITINASE COMPLEX IN ARABIDOPSIS

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Ubiquitination involves the attachment of one or more ubiquitin molecules to a protein to mark it for proteasomal degradation or to alter its activity. Deubiquitinase enzymes reverse ubiquitination by removing attached ubiquitin from tagged proteins. We have identified two Arabidopsis WD40-repeat proteins, encoded by *Lateral Root Stimulator 1* (*LRS1*) and *At2g37160*, that are apparent orthologs of the animal deubiquitinase activators, UAF1 and WDR20, respectively. Using transient expression and immunoprecipitation, we show that these plant WD40 proteins co-purify in a ternary complex with the deubiquitinase UBP3. We show that UBP3 alone has deubiquitinase activity *in vitro* but the WDR20-like protein stimulates this activity more than fourfold. A yeast two-hybrid screen revealed LRS1-interacting proteins that function in hormone signaling pathways. The analysis of a T-DNA insertion mutant demonstrated that LRS1 is required for normal shoot and root development and for signaling in multiple hormone response pathways. These findings indicate that a LRS1/WDR20/UPB3 deubiquitinase complex functions in numerous signaling and developmental pathways in plants.

Poster #6

ANALYSIS OF THE SOYBEAN *(Glycine max)* ROOT *CIS*-REGULOME UPON TREATMENT WITH VARIOUS PHYTOHORMONES

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Phytohormones are plant hormones that regulate virtually all aspects of plant development from seed germination to foliar differentiation. Among the most well-studied roles that phytohormones play is their ability to regulate hormone-driven signaling during the stress response. Host regulatory proteins known as transcription factors (TFs) mediate such signaling by binding onto nearby *cis*-segments of non-coding DNA known as TF binding sites (TFBSs). Thus, quantifying the host *cis*-regulome upon treatment with phytohormones could provide a glimpse into the host regulatory machinery in the event of stress perception. To quantify the soybean *cis*-regulome during defense response, we treated soybean roots independently with four phytohormones involved in pathogen response: salicylic acid, jasmonic acid, ethylene, and auxin. We subsequently sequenced the soybean root transcriptome using RNA-Sequencing (RNA-Seq), producing 200 million paired-end reads across all treatments. Differential expression analysis revealed a set of approximately 29,000 transcripts deemed differentially expressed (DE) in at least one treatment. From this set, a subset of 4,850 transcripts were DE in 3 of the 4 treatments. This subset contained numerous transcripts involved in defense response: PR1, PR10, and NBR-LRR. Statistical analysis of abundant Gene Ontology (GO) Processes revealed "Defense Response" and "Response to oxidative stress" as the most statistically significant terms within this subset.
To investigate magnitude of TFBS over-representation in each of the phytohormone treatments, the top 1,500 differentially expressed genes were identified and their promoter sequences 2.5kb upstream from the transcription start site was extracted. We identified a set of 50 TFBSs rendered over-represented in at least one of the four treatments. TFBSs of TFs perceived in defense response (ZAP1, bZIP910, AtMYB46) were generally over-represented in salicylic acid and jasmonic acid treatments.

Poster #7

EFFECTS OF ELEVATED TEMPERATURE ON CAROTENOID BIOSYNTHESIS IN THE REPRODUCTIVE ORGANS OF THE DIPLOID STRAWBERRY, *FRAGARIA VESCA*

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Carotenoids are tetraterpenoid lipophilic pigments found in most plant organs. In leaves, carotenoids carry out two major functions during photosynthesis: they serve as light harvesting complexes that absorb and dissipate excess light energy, and they are responsible for protection against reactive oxygen species generated in the chloroplast. Carotenoids are also precursors for the production of signaling compounds such as abscisic acid and strigalactone. In flowers and fruits, the bright yellow to red colors of carotenoids are considered to play roles as visual attractants for pollinators and seed dispersers, although roles as antioxidants in these organs are also probable. In flowers of many plants in the Rosaceae family, the anthers and pollen, as well as the carpels are yellow due to the presence of carotenoids, but the precise role(s) of these pigments have not been defined. In strawberry (*Fragaria* species), abiotic stress damage becomes evident during periods of elevated temperatures, when fertilization does not occur and fruit are not formed or develop abnormally. This poor fertilization is often attributed to elevated temperature effects on pollen formation and performance.
Flowers of the diploid woodland strawberry, *F. vesca,* have yellow-orange anthers, yellow carpels, and yellow pollen. HPLC profiling of carotenoids in developing *F. vesca* carpels and stamens revealed that, unlike leaves, the reproductive tissues have no detectable beta-carotene. To investigate this further, candidate genes involved in carotenoid biosynthesis were identified in the *F. vesca* genome, revealing that, unlike what is found in Arabidopsis, several of these genes exist as small gene families. Gene expression patterns throughout the plant, and expression in response to heat stress in the reproductive structures were analyzed. Further studies to determine if expression changes are correlated to carotenoid quantity and quality are underway.

Poster #8

THE ROLE OF RTE1 AND CYTOCHOME B5 IN ETR1 ETHYLENE RECEPTOR FUNCTION

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Arabidopsis has five homologous ethylene receptors that perceive the ethylene signal at the endoplasmic reticulum (ER). One of these receptors, ETR1, associates with a novel integral membrane protein called REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1). Genetic evidence indicates that RTE1 is required for the formation of a functional ETR1 receptor, whereas the other ethylene receptors in Arabidopsis do not require RTE1. To uncover the mechanism by which RTE1 specifically activates ETR1, we screened for RTE1-interacting proteins using the yeast split-ubiquitin assay. The screen yielded an ER membrane-localized isoform of cytochrome *b5* (a heme-binding protein that carries out electron transfer). We subsequently found two additional cytochrome *b5* isoforms that can associate with RTE1. Notably, mutants of all three isoforms have ethylene response defects similar to that of *rte1*. We also discovered that RTE1 homologs can bind heme *in vitro*, raising the possibility that RTE1 carries out redox with cytochrome *b5*s. Interestingly, we determined that a unique proline residue (P9), conserved only in ETR1 orthologs, is largely responsible for the

specificity of RTE1 for ETR1; introduction of P9 into the Arabidopsis ERS1 ethylene receptor was sufficient to convert ERS1 into an RTE1-dependent receptor. Since P9 sits close to the conserved disulfide bond-forming cysteines (C4, C6) in the ETR1 homodimer, we speculate that the P9 side chain interferes with proper folding of the ETR1 homodimer. Taken together, our results suggest a model in which RTE1, together with cytochrome *b*5, promotes the active conformation of ETR1 through oxidative folding.

Poster #9

BACTERIOPHAGE ENDOLYSIN PRODUCTION IN *NICOTIANA BENTHAMIANA* PLANTS

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The increasing spread of antibiotic resistant microorganisms is a growing concern for both modern animal husbandry and medicine. In recent years, peptidoglycan hydrolases (lysins) have acquired significant attention in the fight against bacterial diseases. The main advantages of lysins versus antibiotics are: 1) exogenous lysin application leads to rapid lysis of the bacterial cell wall avoiding such intracellular resistance mechanisms as efflux pumps, thereby making microbial resistance development difficult and 2) endolysins possess narrow species specificity, without effecting aboriginal (normal) microflora. In an effort to examine the antimicrobial efficacy of a Gram-negative phage endolysin, we attempted to produce the endolysin encoded by a cryptic prophage CP-933P [*Escherichia coli* O157:H7 str. EDL933] (GenBank Acc.# NP_287988). The endolysin gene was subcloned into the pET21a inducible prokaryotic expression vector and introduced into *E.coli* strains BL21 (DE3), C43 (DE3) pLysS, C43 (DE3), C41 (DE3) pLysS and C41 (DE3). Expression of the *cp933* gene in bacterial cells led to growth inhibition and lysis of the host cells (strains BL21 (DE3), C43 (DE3) pLysS, C41 (DE3) pLysS) or production of trace amounts of the CP933 endolysin (strains C43 (DE3) and C41 (DE3)). To overcome the expression problems in *E. coli*, we attempted to produce the lysin in *Nicotiana benthamiana* tobacco plants using a *Potato virus* X (PVX)-based transient expression vector. Cytoplasmic expression of *cp933* resulted in death of the apical region of experimental plants 10 days after viral transcript infection. To protect plants against the detrimental impact of CP933, the *cp933* gene containing a 6xHis-tag at its C-terminus was fused at its N-terminus to an N-terminal signal peptide from potato proteinase inhibitor I (PPI-I) to direct CP933 to delta type vacuoles. Plants producing PPI-I/CP933 fusion protein did not exhibit the severe toxic effect observed with cytoplasmic expression of *cp933*. At this stage of our studies, the results of our experiments demonstrated that targeting of proteins to the delta vacuoles is a promising approach for the production of proteins that exhibit toxicity when expressed in plant cells.

Poster #10

POTATO SPINDLE TUBER VIROID DETECTION IN PHLOEM EXUDATES AND GUTTATION FLUID OF TOMATO PLANTS (*SOLANUM LYCOPERSICUM*)

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Potato spindle tuber viroid (PSTVd) is a single-stranded, non protein-encoding, covalently-closed circular RNA molecule (359nt) that infects many horticultural and agricultural crops. PSTVd is mechanically transmitted, replicates in the nucleus, and moves cell-to-cell through plasmodesmata. Though it was well established that systemic spread of PSTVd occurs through the phloem sieve elements and was confirmed by modern genetic and microscopic analyses, there is a lack of the reliable data regarding PSTVd detection in xylem cells. In our study, the possible presence of PSTVd RNA in xylem sap was investigated by analysis of guttation fluid (GF) collected from viroid-infected tomato leaves. Our results revealed the absence of PSTVd in GF as confirmed by RT-PCR analysis. Thus, we can conclude that long distance trafficking of PSTVd through tomato plants does not involve xylem vessels. In addition, we applied a modified method based on an EDTA-mediated phloem exudation technique for detection of PSTVd in phloem exudates of tomato plants. Sequencing, RT-PCR and Southern blot analyses demonstrated the presence of viroid RNA in phloem exudates. To our knowledge, this is the first report of PSTVd RNA detection in phloem exudates obtained by the EDTA-mediated exudation technique, which proved to be a simple, inexpensive and reliable method for phloem exudate analysis. These results suggest the potential application of the EDTA-mediated exudation technique and GF analysis for nucleic acid (including signaling RNAs) isolation, and pathogen detection exclusively in phloem or xylem sap, respectively.

Poster #11

PHOSPHORYLATION IN ETHYLENE SIGNALING: REGULATION OF THE EIN2 PROTEIN

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The plant hormone ethylene mediates numerous aspects of growth and development. Genetic analysis has identified a number of critical elements in ethylene signaling, but it has remained unknown how these elements interact biochemically to transduce the signal from the ethylene receptor complex, which is located at the endoplasmic reticulum (ER) membrane, to transcription factors within the nucleus. The primary gap in our understanding was how the ethylene signal is relayed from the CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) Raf-like protein kinase (Kieber et al., 1993) to ETHYLENE-INSENSITIVE2 (EIN2), an ER membrane-localized Nramp homolog (Alonso et al., 1999), and then to the nucleus in Arabidopsis. To identify possible CTR1 kinase substrates, we used liquid chromatography-tandem mass spectrometry (LC-MS) to uncover proteins that are differentially phosphorylated in the microsomal fraction of etiolated seedlings treated with and without ethylene. EIN2 peptides were detected in both samples, and interestingly, several C-terminal EIN2 peptides were phosphorylated in untreated but not ethylene-treated samples (Chen et al., 2011). Consistent with the fact that CTR1 is active only in the absence and not the presence of ethylene, we found that CTR1 interacts with and phosphorylates six conserved serine and threonine residues in the cytosolic C-terminal domain of EIN2 (Ju et al., 2012). Alanine substitutions that simultaneously block phosphorylation at Serine645 and Serine924 result in the constitutive nuclear localization of the EIN2 C terminus. The Serine924 to alanine substitution alone confers constitutive activation of ethylene responses in *Arabidopsis,* whereas the Serine645 to alanine substitution alone does not (Ju et al., 2012). These results indicate that phosphorylation of EIN2 by CTR1 prevents EIN2 from signaling in the absence of ethylene, whereas inhibition of CTR1 upon ethylene perception is a signal for the cleavage and nuclear localization of the EIN2 C terminus, allowing the ethylene signal to reach the downstream transcription factors. Current work is aimed at uncovering the roles that each of the phosphorylation sites may play in preventing EIN2 cleavage and translocation to the nucleus, as well as EIN2's function in the nucleus. Although EIN2 turnover has been reported in the absence of ethylene treatment (Qiao et al. 2009), this turnover might be unrelated to the phosphorylation status of EIN2. Additionally, we have found that EIN2 can physically interact with itself, and we are investigating whether this interaction is affected by EIN2 phosphorylation. We are also examining proteomic responses in the nucleus in the presence and absence of ethylene treatment, including the phosphorylation status of the ethylene transcription factor EIN3. This work shows how proteomic approaches have significantly advanced our understanding of posttranslational modification in ethylene signal transduction.

Poster #12

FVGD: AN ONLINE RESOURCE FOR STRAWBERRY (*FRAGARIA VESCA*) GENOMIC DATA

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http://bioinformatics.towson.edu/mapmbs/default.aspx

Fragaria vesca, commonly known as the alpine or woodland strawberry, is a versatile experimental plant system that is an emerging model for the Rosaceae family. *F. vesca* shares several features with the other commercially important members of the Rosaceae family such as commercial strawberry (*Fragaria ananassa*), peach and others. RNA from *F. vesca* - YW5AF7 were extracted from different stages of fruit development, and used to profile the transcriptomes. All RNA samples were sequenced using Illumina-based Hisq2000. Library sizes ranged from 12 to 40 million single-ended reads. The reads were aligned to *F. vesca* genome v1.1 build and gene predictions v1.0 build. Gene expression analysis was done on the read counts to find differentially expressed genes. To store the huge amounts of data, and to make searching\mining the data easier for researchers in the field, we developed the online database "*Fragaria vesca* Genomic Database" (FVGD). The database contains samples descriptions, samples statistics, gene annotation, gene expression and gene differential expression analysis. All these information can be accessed publically from a web-based interface. FVGD website provides the users a user friendly search and browse capabilities for all the data stored in the database. Users are able to search for genes using their IDs or their description. Search results can be downloaded in a tabular format so that users can use it on their own computers. We also provide a genome browser (GBrowse) capability to allow researchers to visually see where reads from a given sample were aligned to the genome of *F. vesca*. RNA-Seq analysis stored in the FVGD database is an important resource for researchers investigating fruit development in the Rosacea family and plants in general. The database can be accessed from URL: http://bioinformatics.towson.edu/strawberry/

Poster #13

IDENTIFICATION AND CHARACTERIZATION OF RECEPTACLE FRUIT-SPECIFIC GENES AND PROMOTERS IN *FRAGARIA VESCA*, A DIPLOID STRAWBERRY

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Fragaria vesca, the woodland strawberry, is emerging as a model system for both the commercial, octoploid strawberry and the *Rosaceae*, a family that contains many economically important fruit crops. *F. vesca*, a diploid species, has a small and sequenced genome (240 Mb), is amenable to transformation, has a short life cycle, and is easily grown in a lab setting. The edible, fleshy fruit is unique in that it forms from the floral receptacle and contains two distinct tissues: the pith and cortex.
Previously, our lab generated a two-dimensional (spatial and temporal) transcriptome dataset via RNA-seq to profile strawberry flower and early stage fruit development before and after fertilization. In response to the need for receptacle fruit-specific promoters in strawberry research, we used MATLAB to filter the approximately 34,000 genes of the transcriptome dataset and screen for genes that have transcript expression specifically in cortex and pith tissues. We identified 67 such genes.
Using a subset of the 67 genes, we are working to transform *F. vesca* with *fruit-specific promoter::GUS* constructs to verify transcript expression and specificity in receptacle fruit tissue. We have also conducted bioinformatic analyses to identify motifs common to the promoters of the 67 receptacle fruit-specific genes. Additionally, we have conducted a GO

term analysis to characterize the 67 genes and plan to further study the biological functions of a subset of the genes. Since strawberry receptacle fruit is unique, these fruit-specific genes may shed light on novel developmental processes.

Poster #14

INVESTIGATING THE FUNCTION OF THE N-TERMINAL DOMAIN OF EIN2, AN NRAMP HOMOLOG, IN ETHYLENE SIGNALING IN ARABIDOPSIS

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The gaseous plant hormone ethylene regulates numerous aspects of growth and development. Ethylene responses require the C-terminal domain of a key regulator, EIN2 (ETHYLENE INSENSITIVE2). Recent findings have revealed that phosphorylation and nuclear translocation of a portion of the EIN2 C-terminal domain control ethylene signaling from the ER membrane to the nucleus (Ju et al., 2012, *PNAS* 109: 19486; Qiao et al., 2012, *Science* 338: 390; Wen et al., 2012, *Cell Res.* 22: 1613). The EIN2 N-terminal domain contains 12 predicted transmembrane domains, localizes to the ER membrane, and has sequence homology to Nramp metal ion transporters, yet its function and relation to ethylene signaling remain elusive. Here we find that a novel missense mutation in an invariant residue of a conserved Nramp metal transport motif in EIN2 confers ethylene insensitivity in Arabidopsis. This mutation suggests that the EIN2 N-terminus regulates ethylene signaling via the C-terminus, and raises the possibility that metal transport may be involved in ethylene signaling, since mutating this particular residue disrupts metal transport in *E.coli* (Haemig and Brooker, 2004, J. *Memb. Biol.* 201:97). The mutation does not affect EIN2 subcellular localization, suggesting that folding is not grossly altered. Currently we are using *E. coli* to investigate whether EIN2 plays a role in metal transport. This preliminary data is perhaps the first evidence that the N-terminal domain of EIN2 plays a role in ethylene signaling, and further characterization of this mutant allele may help to decipher its function. Since EIN2 has been isolated from numerous genetic screens, it is possible that gaining a better understanding of EIN2 will not only advance a mechanistic understanding of ethylene signaling, but allow us to gain a better understanding of crosstalk between ethylene and other signaling pathways.

Poster #15

ECTOPIC EXPRESSION OF *AtPAD4* BROADENS RESISTANCE OF SOYBEAN TO SOYBEAN CYST AND ROOT-KNOT NEMATODES

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Plant parasitic nematodes are obligate biotrophic parasites generally attacking the roots of many plant species. They have a wide host range and can have adverse effects on the yield of crop plants by damaging the crops either directly or as viruses vectors. The worldwide annual crop losses caused by plant parasitic nematodes have been estimated at 157 billion dollars. Several economically important species are pathogens of different crop plants and the cyst and root-knot nematodes within the family *Heteroderidae* are among the most important. They are obligate endoparasites of plant roots which they enter as second stage juveniles (J2 larvae) and establish specialized feeding structures. The gene encoding *PAD4* [PHYTOALEXIN-DEFICIENT4] is required in *Arabidopsis* for expression of several genes involved in the defense response to *Pseudomonas syringae* pv. *maculicola*. *AtPAD4* [*Arabidopsis thaliana PAD4*] encodes a lipase-like protein that plays a regulatory role mediating salicylic acid signaling. We expressed the gene encoding *AtPAD4* in soybean roots of composite plants to test the ability of *AtPAD4* to deter plant parasitic nematode development. The transformed roots were challenged with two different plant parasitic nematode genera represented by soybean cyst nematode [SCN; *Heterodera glycines*] and root-knot nematode [RKN; *Meloidogyne incognita*]. Expression of *AtPAD4* by soybean roots decreased the number of mature SCN females 35 days after inoculation by 68 percent. Similarly, soybean roots expressing *AtPAD4* exhibited 77 percent fewer galls when challenged with RKN. This work provides a basis for unraveling the potential role of defense signaling genes in quantitative disease resistance in this major crop species, and it demonstrates that an *Arabidopsis* gene can confer resistance in an important field crop to two genera of nematodes having worldwide importance.

Poster #16

ARABIDOPSIS TSO1 REGULATES MALE FLORAL ORGAN DEVELOPMENT AND MICROGAMETOGENESIS

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The Arabidopsis TSO1 protein is a key regulator of both cell proliferation and floral organ differentiation. *tso1-1* mutant plants show fasciated inflorescence meristems, develop callus-like structures instead of normal floral organs, and are sterile. TSO1 encodes a putative transcription factor containing two conserved CXC domains known to be involved in DNA binding, and may be part of a conserved chromatin complex (called the dREAM complex in *Drosophila*) that regulates cell cycle, development, and reproduction. To identify regulatory targets of TSO1, RNA-Seq analysis of *tso1-1* plants carrying an inducible TSO1-GR system was performed. Several genes with roles in anther tapetum development were identified as putative targets of TSO1, including *AMS*, *MYB103/MS188*, *MYB99*, and *SHT*. Additional genes identified by RNA-Seq include *NAC025*, *XYLP8*, *KCS15*, *GH3-16*, and *ATA27*, all of

which are specifically expressed in stage 10-11 flowers or later-stage anthers, as well as the *SADHU6-1* noncoding retrotransposon. These results are supported by previous microarray analyses in which a large number of genes involved in pollen development were found to be suppressed in *tso1-1* mutants. qRT-PCR was carried out to verify the results of the RNA-Seq experiment, and chromatin immunoprecipitation experiments are underway to assay for TSO1 interaction with these putative target genes.

As the majority of putative TSO1 targets identified thus far are involved in anther and pollen development, we further characterized the role of TSO1 in these processes. We examined pollen phenotypes in the *tso1-3* mutant. Around 50% of *tso1-3* pollen are collapsed and do not contain DNA, while the remaining 50% are highly variable in size, with both abnormally large and small pollen observed. Many *tso1-3* tetrads contain greater or fewer than the normal four microspores. Taken together, these results indicate that TSO1 may directly regulate the expression of genes required for microsporogenesis and tapetal development.

Poster #17

DETECTION AND IDENTIFICATION OF PLANT VIRUSES BY MICROARRAY

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Because a variety of plant viruses pose a significant threat to food security, agricultural production and ornamental flora, a rapid assay that can detect known and even uncharacterized viruses is required. To address this concern, we are developing a microarray-based assay for the identification and categorization of plant viruses. The Universal Plant Virus Microarray (UPVM) is composed of 10,000 elements covering every taxon/node of the taxonomic tree for all plant viruses available in GenBank. Detection of low-titer viruses on the UPVM has been a significant challenge. To overcome this problem we have employed subtractive hybridization to remove high copy RNA (primarily ribosomal RNA) from total RNA samples. Currently we are validating the UPVM on a wide variety of samples and optimizing sample processing methods. Here we present a sample of our results.

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