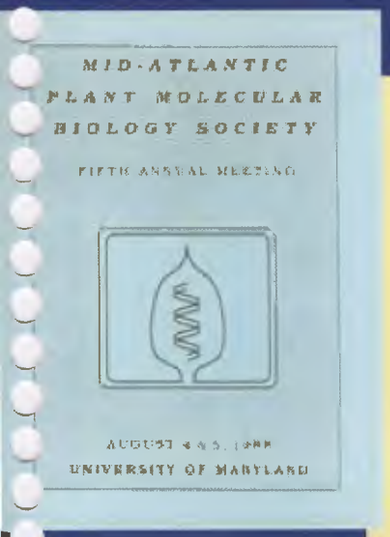
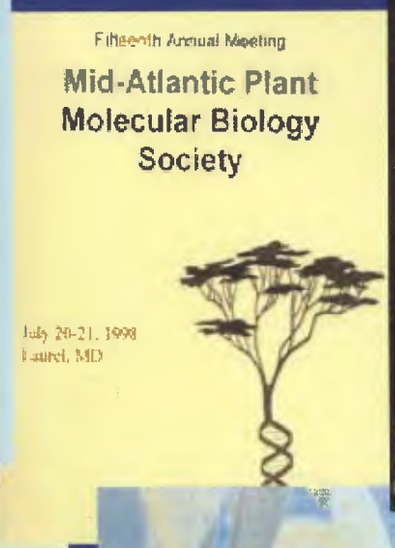
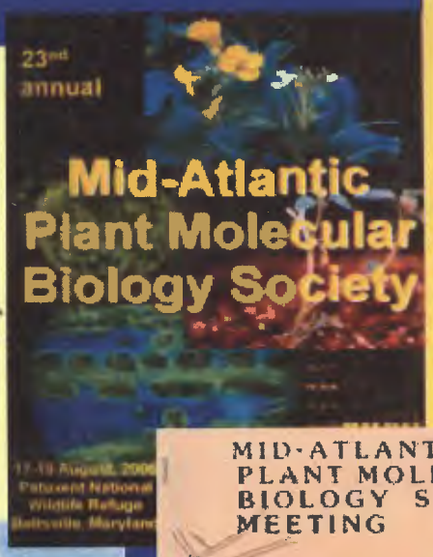
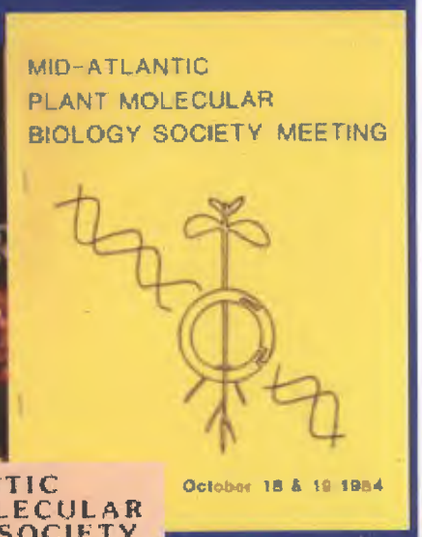
25TH ANNIVERSARY mapmbs

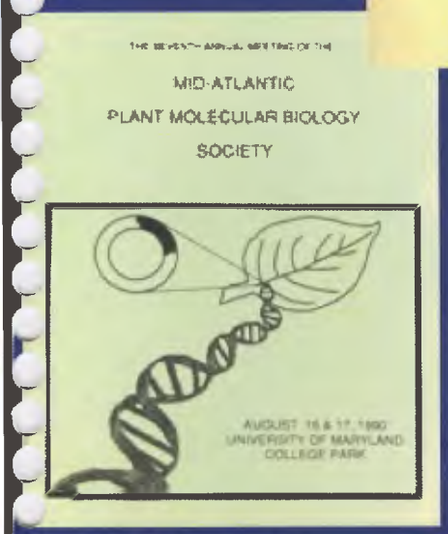
August 21-22, 2008
The Great Room at Savage Mill
Savage, MD

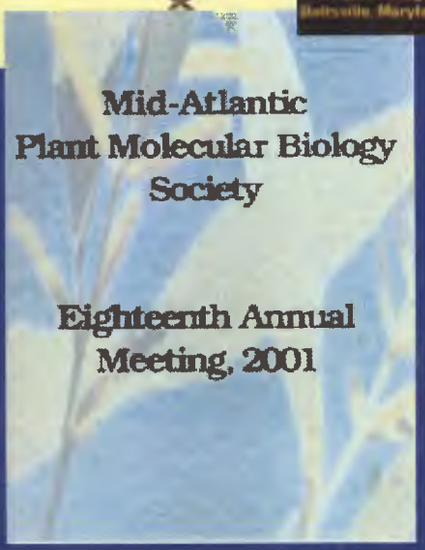


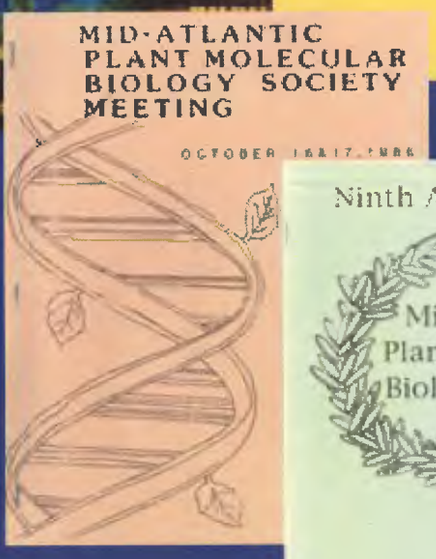


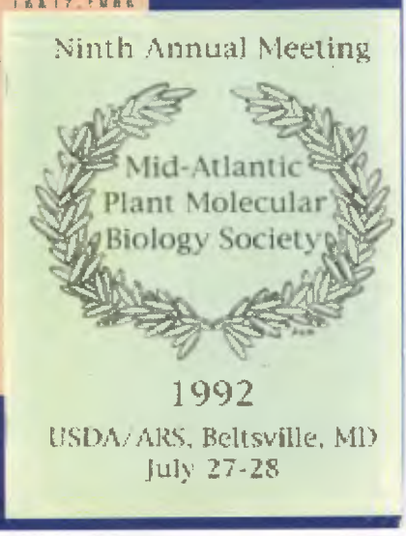












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ORGANIZING COMMITTEES

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Ben Matthews
Bret Cooper
Savithiry Natarajan
Janet Slovin
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Dilip Laksman
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COVER DESIGN, Liz Matthews (logo) and Leslie Wanner using old MAPMBS cover illustrations provided by Sue Mischke

INTRODUCTION

On behalf of the Organizing Committees we welcome you to the twenty-fifth meeting of the Mid-Atlantic Plant Molecular Biology Society, MAPMBS 2008. MAPMBS was formed a quarter of a century ago to provide a high quality, accessible and affordable plant molecular biology meeting each year for scientists in the Mid-Atlantic region. Our goal continues to be to bring some of the best minds in the country to the Mid-Atlantic region to present interesting advances in plant molecular biology at a reasonable price and at an accessible location. We hope to encourage students, post-docs, and senior scientists to actively participate in presentations and discussions. In addition, the meeting is designed to promote interaction among scientists in an informal atmosphere. Therefore, we provide on-site lunches and breaks so that each participant has the opportunity to meet invited speakers and other presenters.

As you know, the meeting encompasses a broad range of research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests. Please contact members of the organizing committee if you have any thoughts or comments for consideration in the planning of future meetings. Also, if you are interested, please join next year's organizing team and volunteer your services to improve upon this year's meeting. All are welcome at any stage of the planning and organizing process.

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

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society.

Benjamin F. Matthews and Leslie A. Wanner

Co-chairs

A little history of the MAPMBS
by Sue Mischke

Plant molecular biology came to be recognized as a consequential discipline in the early 1980's. The first U.S. Gordon Conference on Plant Molecular Biology was convened in 1980. The cover date for volume 1(1) of the journal "Plant Molecular Biology" is March 1981 and the issue was a mere 79 pages. Volume 1(2) was dated more than a year later, (June 1982) and added another 83 pages to the literature. "Biotechniques," the popular free journal, emerged in 1983.

The October, 1985 issue of BioEssays (vol 3, #4) was devoted to plant molecular biology, and celebrated the formation of the ISPMB. Leon Dure's introductory editorial for the BioEssays issue said of plant molecular biology: "Ten years ago this field did not exist as an identifiable sub-discipline of biochemistry and genetics, nor did its practitioners consider themselves members of a recognizable club." The dedication of the issue by Richard Flavell explained that the "new interest in plant molecular and cell biology has led to the formation of a new society; the International Society for Plant Molecular Biology, which is responsible for the first-ever International Congress of Plant Molecular Biology, being held this October at Savannah, Georgia."

The excitement attending the formal emergence of this new discipline was celebrated by ARS administrators (who, among other things, expanded the name of the Beltsville lab devoted to plant tissue culture to the "Tissue Culture and Molecular Genetics Laboratory") and ARS scientists (who salivated over the program of the upcoming International Congress in Savannah). It was obvious that, given the perpetual budget crunch imposed upon ARS, it would be impossible for all the interested scientists to attend this historic meeting. A small group of scientists ultimately came to the conclusion that the only way to guarantee they could attend a plant molecular biology meeting with a top-notch venue of speakers was to put it on themselves.

Beginning in the spring, a meeting was planned for October, 1984. Scientists from a variety of labs (Tissue Culture and Molecular Genetics, Soilborne Diseases, Plant Hormone, Tobacco Lab and others) were recruited to help put on the meeting, speakers in town for grant review panels were enticed to contribute their wisdom; companies were offered space to exhibit their goods for a small fee. Typewritten abstracts were submitted and xeroxed for the proceedings booklet and, when the registration data tape on the Wang computer broke, name tags were assembled from information recovered from 3x5 cards. The mindset was strictly low-budget and the registration fee was $5 – payable in advance, or at the door. (A few of the very senior scientists arrived at the door, expecting their stature to relieve them of any registration cost, but the gatekeepers stood firm.)

Originally planned for the BARC auditorium, the meeting was hurriedly moved to the National Arboretum when a conflict arose with renovation plans for the auditorium. Since nearby restaurants were not available, Jim Saunders collected $2 from all the participants, and picked up drinks, cold cuts and other makings for lunch. Everyone assembled his own sandwich, and we enjoyed a gorgeous fall day on the patio of the Visitors Center.

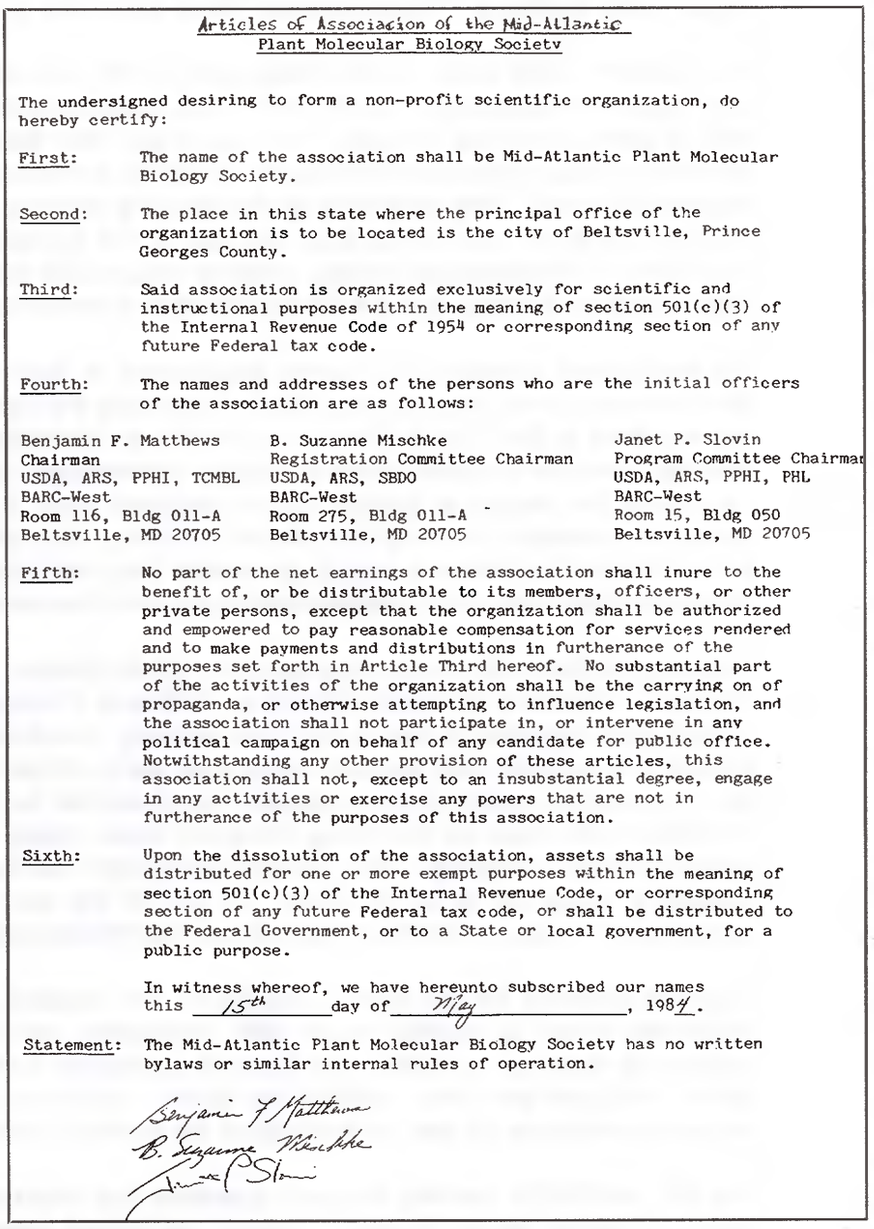
The first MAPMBS meeting featured speakers that included Charles Arntzen, Joachim Messing and Ted Diener among others. Among the invited speakers for subsequent meetings are a veritable parade of NAS members and other well-known scientists, including Roger Beachy, Bob Goldberg, Olen Yoder, Gloria Corruzi, Marvin Edelman, Brian Larkins, Ian Sussex, Peter Quail, Russell Malmberg, David Ho, Richard Dixon, Anthony Cashmore, Chris Lamb, Eugene Nester, Virginia Walbot, Andrew Bent, Daniel Klessig, Jen Sheen, Michael Thomashow, Greg Martin, Phillip Rea, Roger Innes and many others.

Ben Matthews' introduction to the abstract booklet of the initial MAPMBS meeting in 1984 explained that the "society was formed to provide a forum devoted to the exchange of ideas and

information concerning plant molecular biology. Hopefully, the society will ensure scientists in the Mid-Atlantic region of a high quality accessible and affordable plant molecular biology meeting each year. … If the society is to continue, participation by members in running the society and meeting is needed. This society is extremely flexible; we have no ground rules. Therefore, everyone is encouraged to help out and provide advice and personal views…"

Many things have changed, but the purpose and structure of MAPMBS have not. MAPMBS has lived up to the high standards we envisioned in 1984. Indeed, we have outlasted the ISPMB, which disbanded after the 8th International Congress in 2006. (However, there will be a 9th IPMB International Congress in 2009, with the deletion of "Society" from the name, and it is apparently following our model of volunteer sponsorship for the meeting.)

The annual meeting has been convened in many locations, with several different organizers, but the continuing thread – nay, the spirit and spine of MAPMBS – has been Ben Matthews, who has shouldered much of the responsibility since its inception. We thank you, Ben. It has been a great quarter-century!



Articles of Association of the Mid-Atlantic
Plant Molecular Biology Society

The undersigned desiring to form a non-profit scientific organization, do hereby certify:

First: The name of the association shall be Mid-Atlantic Plant Molecular Biology Society.

Second: The place in this state where the principal office of the organization is to be located is the city of Beltsville, Prince Georges County.

Third: Said association is organized exclusively for scientific and instructional purposes within the meaning of section 501(c)(3) of the Internal Revenue Code of 1954 or corresponding section of any future Federal tax code.

Fourth: The names and addresses of the persons who are the initial officers of the association are as follows:

Benjamin F. Matthews	B. Suzanne Mischke	Janet P. Slovin
Chairman	Registration Committee Chairman	Program Committee Chairman
USDA, ARS, PPHI, TCMBL	USDA, ARS, SBDO	USDA, ARS, PPHI, PHL
BARC-West	BARC-West	BARC-West
Room 116, Bldg 011-A	Room 275, Bldg 011-A	Room 15, Bldg 050
Beltsville, MD 20705	Beltsville, MD 20705	Beltsville, MD 20705

Fifth: No part of the net earnings of the association shall inure to the benefit of, or be distributable to its members, officers, or other private persons, except that the organization shall be authorized and empowered to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of the purposes set forth in Article Third hereof. No substantial part of the activities of the organization shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the association shall not participate in, or intervene in any political campaign on behalf of any candidate for public office. Notwithstanding any other provision of these articles, this association shall not, except to an insubstantial degree, engage in any activities or exercise any powers that are not in furtherance of the purposes of this association.

Sixth: Upon the dissolution of the association, assets shall be distributed for one or more exempt purposes within the meaning of section 501(c)(3) of the Internal Revenue Code, or corresponding section of any future Federal tax code, or shall be distributed to the Federal Government, or to a State or local government, for a public purpose.

In witness whereof, we have hereunto subscribed our names this 15th day of May, 1984.

Statement: The Mid-Atlantic Plant Molecular Biology Society has no written bylaws or similar internal rules of operation.

Benjamin F. Matthews
B. Suzanne Mischke
Janet P. Slovin

Sponsors and Exhibitors

Company	Representative
Beltsville Agricultural Research Center	Joseph Spence, Area Director
Dupont/Pioneer	Enno Krebbers
Atlantic Technology Group, Inc.	Scott Saveleski, Dan Query
Stratagene/Agilent Corporation	Mini Varughese
Rainin Instrument	Jessica Oliver
CLP	Jacqueline G. Toti
Promega	Dongmei (May) Guo
Bio- Rad	Kimberly Swift
Pressure Biosciences	Roxana McCloskey
Carl Zeiss MicroImaging	Jennifer Malloy
IDT	John Wilhelm III
Tecan	Tom Hill
Roche	Steve Martin, Karen Fredricksen, Kerry English
Daigger	Marc Friedman
Qiagen	Marcia Armstrong
Edge Bio	Doreen Dyer
Fermentas Life Sciences	Sandra Jabra

2008 MAPMBS 25th ANNIVERSARY MEETING SCHEDULE

Thursday, August 21

8:30 Registration and poster set-up
9:00 Welcome

Session I Science Policy Issues
Moderator, Bret Cooper, USDA-ARS Beltsville, MD

9:05 **E. William Colglazier**, National Academy of Sciences and National Research Council, Washington DC
Science policy and its evolution over twenty five years

9:55 **Chris Wozniak**, EPA, Office of Pesticide Programs, Washington DC
EPA regulation of pesticidal substances expressed in plants

10:30 Break -- coffee/exhibitors/posters

10:55 **Enno Krebbers**, DuPont/Pioneer Crop Genetic Research, Wilmington DE
Why can't we all just get along? Difficulties in private-public sector collaborative relationships

Session II Pathogens/Stress
Moderator, Chris Dardick, USDA-ARS Appalachian Fruit Research Kearnysville, WV

11:45 **Dumitru Macarisin**, USDA-ARS Appalachian Fruit Research Kearnysville, WV
Proteomic Analysis of drought resistance of in crabapple seedlings primed by the xenobiotic β-aminobutyric acid

12:05 **Hua Lu**, University of Maryland Baltimore County, Baltimore MD
Identification and characterization of *acd6-1* SUPRESSOR 6, a novel defense gene in *Arabidopsis*

12:30 **LUNCH/posters/exhibitors**

1:40 **Alan Collmer**, Cornell University
Pseudomonas syringae and plants: a relationship built on lethal injections

Session III Genomics/Gene Regulation
Moderator: Shannon Alford, Virginia Technical University

2:30 **Dan Bell**, University of Maine and USDA-ARS Beltsville, MD
Estimates of spatial genetic structure within and among clones of wild lowbush blueberry in Maine using EST-PCR molecular markers

2:50 **Jeanne Wilson**, DuPont/Pioneer Crop Genetic Research, Wilmington DE
Confirming and characterizing microRNAs through expression profiling
;
3:40 Break -- coffee/exhibitors/posters

4:05 Introduction of keynote speaker: **Ben Matthews**, USDA-ARS

4:10 **Keynote speaker**: **Maureen R. Hanson**, Cornell University

Dynamic Organization of the Living Plant Cell

Friday, August 22

Session IV Plant Hormones/Signaling
Moderator, Caren Chang, University of Maryland College Park

9:00 **Wan-Ling Chiu**, Virginia Commonwealth University, Richmond, VA
MKP1 enhances cytokinin sensitivity, promotes early flowering and floral meristem longevity in tobacco plants

9:20 **Peter McCourt**, University of Toronto
A role for strigolactones in Arabidopsis: A new plant hormone

10:10 **Jen Sheen**, Harvard University
Cytokinin signaling circuitry and stem cell control

10:55 Break -- coffee/exhibitors/posters

11:20 **Xiao-Ning Zhang**, Dept Cell Biology and Molecular Genetics, University of Maryland
The splicing factor gene SR45 functions through both splicing isoforms in Arabidopsis thaliana

11:40 **Zhiyong Wang**, Carnegie Institution, Stanford University
Brassinosteroid signal transduction and regulation of plant development

12:30 Business meeting

12:45 LUNCH/posters/exhibitors

Session III Genomics/Gene Regulation, part 2
Moderator, Arianne Tremblay, USDA-ARS Beltsville, MD

1:30 **Scott Jackson**, Purdue University
Comparative and evolutionary genomics of soybean (Glycine max) and common bean (Phaseolus vulgaris)

2:20 **William D. Hitz**, DuPont Central Research and Development, Wilmington, Delaware
Dupont Biofuels

3:10 **Vincent Klink**, Department of Biological Sciences, Mississippi State University
An investigation of Heterodera glycines (soybean cyst nematode) gene expression and a candidate gene approach to study its development

3:30 **Closing and thank you, *MAPMBS co-chairs***

Please take down your posters

Poster #	Abstract Page	Author(s); affiliation: *TITLE*
01	25	**Aparna Gurugunti,** Jovita Haro, Kymber Nichols, and Benjamin Matthews *University of Maryland University College, 3501, University Blvd East, Adelphi, MD 20783* ***MONSANTO SEQUENCE 3 CONTIG ANNOTATION PROJECT AT USDA***
02	26	**Elitsa A. Ananieva**[1], F. Les Erickson[2], Amanda Ely[2] and Glenda Gillaspy[1] *[1]Department of Biochemistry, Virginia Tech, 111 Engel Hall, Blacksburg, VA; [2]Department of Biology, Salisbury University, Salisbury, MD* ***REGULATORY INTERACTION BETWEEN AN INOSITOL POLYPHOSPHATE 5-PHOSPHATASE AND SNRK1.1 (AKIN10) LINKS INOSITOL, SUGAR AND STRESS SIGNALING***
03	26	**Arianne Tremblay**[1]**,** Shuxian Li[2]**,** Brian E. Scheffler[3], Benjamin F. Matthews[1] *[1]Soybean Genomics & Improvement Laboratory, USDA-ARS, Beltsville, MD [2]USDA-ARS, CGPRU, Stoneville, MS 38776 and [3]USDA-ARS, MSA Genomics Laboratory, Stoneville, MS 38776* ***PHAKOPSORA PACHYRHIZI GENE EXPRESSION DURING INFECTION IN SOYBEAN***
04	27	**Lyndel W. Meinhardt**[1], Bryan A. Bailey[1], Mark Guiltinan[2] and Marcelo F. Carazzolle[3], Gustavo Lacerda[3], Jorge Mondego[3], Eduardo Formigheri[3] and Gonçalo A.G. Periera[3] *[1]Sustainable Perennial Crops Laboratory, USDA–ARS Beltsville; [2]Department of Horticulture, The Pennsylvania State University; [3]Laboratório de Genômica e Expressão, Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, São Paulo, Brazil* ***COMPARATIVE GENOME ANALYSES OF MONILIOPHTHORA PERNICIOSA AND MONILIOPHTHORA RORERI: TWO CLOSELY RELATED PHYTOPATHOGENIC BASIDIOMYCETES THAT CAUSE DISTINCTLY DIFFERENT DISEASES OF THEOBROMA CACAO***
05	27	**Dapeng Zhang**[1]**,** Sue Mischke[1], Enrique Arévalo-Gardini[2], Lambert A. Motilal[3], Virupax Baligar[1], Bryan Bailey[1], Luiz Zúñiga-Cernades[2], Carlos E. Arévalo-Arévalo [2] and Lyndel Meinhardt[1] *[1]USDA ARS PSI SPCL, Beltsville, MD; [2]Instituto de Cultivos Tropicales (ICT), Jr. Santa Maria 241, Banda de Shilcayo, Tarapoto, Peru; [3]Cocoa Research Unit (CRU), University of the West Indies, St. Augustine, Trinidad, Republic of Trinidad and Tobago, West Indies* ***DISSECTING GENETIC STRUCTURE IN FARMER SELECTIONS OF THEOBROMA CACAO IN THE PERUVIAN AMAZON: IMPLICATIONS FOR ON-FARM CONSERVATION AND REHABILITATION***
06	28	**Robert Berkey,** Wenming Wang and Shunyuan Xiao, *Center for Biosystems Research, University of Maryland Biotechnology Institute, Rockville, MD 20850* ***FUNCTIONAL CHARACTERIZATION OF THE HOMOLOGS OF THE BROAD-SPECTRUM DISEASE RESISTANCE GENE RPW8 IN ARABIDOPSIS***

07 29 **Wenming Wang,** Xiaohua Yang, Samantha Tangchaiburana, Undral Orgil, Roland Ndeh and Shunyuan Xiao
Center for Biosystems Research, University of Maryland Biotechnology Institute, Maryland, 20850, USA

THE ROLE OF A PUTATIVE INOSITOL PHOSPHORYLCERAMIDE SYNTHASE IN PLANT PROGRAMMED CELL DEATH ASSOCIATED WITH DEFENSE

08 29 **Robert Riggs**[1] and Nadim Alkharouf[2]
[1]Biotechnology Studies Program, Bioinformatics Track, Graduate School of Management and Technology, University of Maryland University College, Adelphi, MD. [2]Dept of Computer and Information Sciences & Faculty Molecular Biology, Biochemistry and Bioinformatics at Towson University, Towson MD

dbEstUMD: BIOINFORMATICS TOOL AUTOMATES SUBMISSION OF EXPRESSED SEQUENCE TAGS TO NCBI'S DBEST DATABASE

09 30 **Christopher A. McClellan,** Mandy D. Kendrick, Chun-Hai Dong, and Caren Chang
Department of Cell Biology & Molecular Genetics, Bioscience Research Building University of Maryland, College Park, MD 20742

NEW MUTANTS WITHIN THE ETHYLENE SIGNALING PATHWAY IN ARABIDOPSIS

10 30 Seok Jun Moon[1], Dongjin Shin[1], Se Youn Han[1], HyeEun Lee[1], Sang Ryeol Park[1], Mi-Jeong Jeong[1], Seong Kon Lee[1,2], Hawk-Bin Kwon[3], Bu Young Yi[4], Soo-Chul Park[1,2], and **Myung-Ok Byun[1]***,
[1]National Institute of Agricultural Biotechnology, Rural Development Administration (RDA), Suweon, Korea; [2]USDA-ARS, Soybean Genomics & Improvement Lab, Beltsville, MD 20705;[3]Department of Biomedical Sciences, Sunmoon University, Asan, 336-708, Korea; [4]Department of Environmental Horticulture, University of Seoul, Seoul 130-743, Korea

MOLECULAR CHARACTERIZATION OF StMyb1 AND StRD22 GENES FROM POTATO PLANT

11 31 **Mi-Jeong Jeong[1]***, Chang-Ki-Shim[1], Seong-Kon Lee[1,2], Beom-Gi Kim[1], Hye-Jin Yoon[1], Woo-Seuk Cho[1], Myeong-Ok Byun[1], Soo-Chul Park[1,2] *[1]Rural Development Administration (RDA), National Institute of Agricultural Biotechnology, Suweon, Korea, [2]USDA-ARS, Soybean Genomics & Improvement Lab, Beltsville, MD 20705*

SOUND STIMULATES GENE EXPRESSION AND PATHOGENS CONTROL

12 31 **Ana M. Bocsanczy[1], John G. Phillips[2], Chris D. Dardick[1], Schuyler S. Korban[3], Carole L. Bassett[1], Michael E. Wisniewski[1] and John L. Norelli[1,4]**
[1]USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430; [2]USDA-ARS North Atlantic Area. 600 E Mermaid Lane, Wyndmoor, PA 19038; [3] Department of Natural Resources & Environmental Sciences, University of Illinois at Urbana-Champaign, 1201 West Gregory Drive, Urbana, IL 61801

USING AN APPLE (MALUS) MICROARRAY FOR EXPRESSION ANALYSIS OF RESPONSES TO COMPATIBLE AND INCOMPATIBLE PATHOGENS

13 32 **Donna A. Lalli,** Timothy S. Artlip, Michael E. Wisniewski and John L. Norelli
USDA-ARS, Appalachian Fruit Research Station, 2217 Wiltshire Rd., Kearneysville,

WV, 25430, USA

TRANSGENIC EXPRESSION OF THE ERWINIA AMYLOVORA (FIRE BLIGHT) EFFECTOR PROTEIN EOP1 SUPRESSES HOST BASAL DEFENSE MECHANISMS IN MALUS (APPLE)

14 33 **Shannon Alford** and Glenda Gillaspy
Biochemistry Department Virginia Polytechnic Institute and State University Latham Hall, Blacksburg, VA

HOW DO PLANTS WITH ALTERED VITAMIN C COPE WITH STRESS?

15 33 **Natasha Safaee,** Jonathan Watkinson and Glenda Gillaspy
Department of Biochemistry Virginia Tech, Blacksburg, VA 24061

A FUNCTIONAL ANALYSIS OF THE PHD-FINGER DOMAIN CONTAINING PROTEINS, ING1 AND ING2, IN ARABIDOPSIS

16 34 **C. Srinivasan**, Ann Callahan, Chris Dardick, Mark Demuth, Dennis Bennett and Ralph Scorza
USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430, USA

ECTOPIC EXPRESSION OF POPLAR FLOWERING LOCUS T1 GENE INDUCED EARLY FLOWERING AND NORMAL FRUIT DEVELOPMENT IN PLUM (PRUNUS DOMESTICA L.)

17 35 **Seong-Kon Lee**[1, 2], So-Hyeon Baek[1, 2], Kyung-Hwan Kim[1, 2], Margaret H. MacDonald[1], Vincent Klink[1], Soo-Chul Park[1, 2], Benjamin F. Matthews[1]
[1]USDA-ARS, Soybean Genomics & Improvement Lab, Beltsville, MD 20705; [2]Rural Development Administration (RDA), Suwon, 441-707 Korea

FUNCTIONAL ANALYSIS OF NEMATODE-RESPONSIVE PR GENES IN SOYBEAN PLANTS

18 35 **William Slade,** Elitsa A. Ananieva, and Glenda Gillaspy
Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061

PROTEIN-PROTEIN INTERACTIONS OF THE INOSITOL POLYPHOSPHATE 5-PHOSPHATASES

19 36 **Kerry F. Pedley**
USDA-ARS, Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Ft. Detrick, MD 21702

A PCR-BASED ASSAY FOR DETECTION OF PUCCINIA HORIANA ON CHRYSANTHEMUMS

20 36 **Christine Prasse**, Maria Smith, Eun-Hyun Rhee, Jeff Elhai, and Wan-Ling Chiu
Department of Biology, Virginia Commonwealth University, Richmond, VA 23284

GUNNERA: A MODEL SYSTEM TO STUDY PLANT-NOSTOC SYMBIOSES

21 37 **Margaret MacDonald,** Vincent Klink, Hunter Beard and Benjamin Matthews
USDA-ARS Soybean Genomics & Improvement Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705, U.S.A

THE EFFECT OF SOYBEAN HAIRY ROOT MORPHOLOGY ON THE INFECTIVITY OF SOYBEAN CYST NEMATODE

22 38 **Heba Ibrahim**[1,2], Susan Meyer[2], Ebtissam Hussein[1] and Benjamin F. Matthews[2]
[1]Genetics Department, Faculty of Agriculture, Cairo University, Giza, Egypt and [2]United States Department of Agriculture, Plant Sciences Institute, Beltsville, MD 20705

POST TRANSCRIPTIONAL GENE SILENCING OF A PLANT PARASITIC-ROOT KNOT-NEMATODE IN SOYBEAN

23 39 **Roland Roberts**[1], Larry Wimmers[1], Andre Wells[1], Sara Campbell[1], Olatejumade Adegbenro[1], Karimat Okanlawon[1], Darin A. Sukha[2], David R. Butler[2], Frances L. Bekele[2], and James A. Saunders[1]
[1]Department of Biological Sciences, 8000 York Road, Towson University, Towson, MD 21252. [2]Cocoa Research Unit, University of West Indies, St Augustine, Trinidad and Tobago, West Indies

DETECTION OF MISIDENTIFIED PLANTS IN INTERNATIONAL COCOA GENEBANK, TRINIDAD

24 39 **Nazia Sabrina Majumder**[1], Sue Tolin[2] and Jim Tokuhisa[1]
[1]Department of Horticulture, [2]Department of Plant Pathology & Weed Science, Virginia Tech, Blacksburg, VA 24061

GLUCOSINOLATES AND THE INFECTION OF ARABIDOPSIS THALIANA WITH TURNIP MOSAIC VIRUS (TuMV)

25 40 **Alice Mweetwa,** Rebecca Poe, and Jim Tokuhisa
Department of Horticulture, Virginia Tech, Blacksburg, VA 24061

BIOSYNTHESIS AND REGULATION OF STEROIDAL GLYCOALKALOIDS IN SOLANUM CHACOENSE

26 41 **Reza Sohrabi** and Dorothea Tholl
Department of Biological Sciences, 427 Latham Hall, Virginia Tech, Blacksburg, VA 24061

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

27 41 **Martha Vaughan** and Dorothea Tholl
Department of Biological Sciences, 427 Latham Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

CONSTITUTIVE AND INDUCED VOLATILE TERPENE FORMATION IN ARABIDOPSIS ROOTS IN INTERACTION WITH THE ROOT HERBIVORE BRADYSIA SPP. (FUNGUS GNAT)

28 42 **Jung-Hyun Huh** and Dorothea Tholl
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VOLATILE TERPENE FORMATION IN ARABIDOPSIS ROOTS IN INTERACTION WITH THE SOIL-BORNE PATHOGEN PYTHIUM IRREGULARE

29 42 Raina Kumar, Margaret MacDonald, Hunter Beard, Dr. Benjamin F. Matthews
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EXPRESSION OF THE CASSAVA VEIN MOSAIC VIRUS (CSVMV) PROMOTER IN TRANSGENIC SOYBEAN (GLYCINE MAX) PLANTS

30 43 Rita Zrenner[1], Heike Riegler[1], Cathleen Marquard[1], Peter R. Lange[1], Claudia Geserick[1], Caren E. Bartosz[2], Celine T. Chen[2], and Robert D. Slocum[2]*
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A FUNCTIONAL ANALYSIS OF THE PYRIMIDINE CATABOLIC PATHWAY IN ARABIDOPSIS

SCIENCE POLICY AND ITS EVOLUTION OVER TWENTY FIVE YEARS

E. William Colglazier

Executive Officer, National Academy of Sciences
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Science Policy includes both (1) "policy for science," which refers to policies relevant for ensuring a strong scientific enterprise and (2) "science for policy," which refers to scientific input relevant for making wise public policy decisions. The first is of great interest to the scientific community as well as important for achieving the goals that motivate government support for science. It includes issues such as government funding for scientific research in various fields, government support for graduate training, mechanisms for awarding research and training funds, priority-setting and assessment of progress in fields, polices for publishing scientific research and assuring the integrity of research data, etc. The second is of great interest to the government and the public, and it offers an opportunity for the scientific community to provide advice and assess current scientific knowledge relevant to policies dealing with national competitiveness, national security, environmental protection, public health, energy, agriculture, etc. Both types of science policy grew greatly in importance in the decades following World War II. Even with the end of the Cold War their evolution over the past 25 years has resulted in growing influence of scientists and scientific input on governmental decisions in the U.S. and in other countries. This talk will trace some of the key themes in this recent evolution and its affect on our nation, the scientific community, and the world.

EPA REGULATION OF PESTICIDAL SUBSTANCES EXPRESSED IN PLANTS

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While we typically think of pesticides as synthetic chemicals sprayed onto plants or other substrates for pest control, substances with pesticidal activity are produced naturally in plants and through the introduction of transgenes from various sources. Pesticidal substances include proteins and metabolites which repel, mitigate, kill or otherwise suppress plant pests. Plant-incorporated Protectants (PIPs) are the pesticidal substances produced from transgene expression *in planta* and include the nucleic acids responsible for their production. PIPs are regulated by the EPA under authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and their presence as pesticidal substances in or on food or feed is regulated by EPA and FDA under the Federal Food, Drug and Cosmetic Act (FFDCA).

The USDA-APHIS also regulates plants genetically modified through rDNA techniques, including those considered as PIPs. Under the Plant Protection Act, APHIS requires that any environmental field releases of PIPs and other GM plants be approved through a permitting process and an environmental assessment. Under FIFRA, EPA requires that an experimental use permit be approved prior to release of a cumulative area of 10 acres / year / pest for terrestrial experimentation and 1 acre cumulative area / year / pest for aquatic testing. The APHIS data submission and review process focuses on potential agro-ecosystem impacts as required by the

PPA and the National Environmental Policy Act, whereas the EPA risk assessment process under FIFRA and FFDCA includes food safety, human health and environmental assessments where PIPs are involved.

When a novel (unregistered) pesticide is applied to or expressed in a food or feed crop, any commodities derived which enter commerce as food or feed are considered as adulterated under FFDCA and can be removed from distribution by the FDA (with associated fines and penalties). Without a food tolerance or exemption from the requirement of a food tolerance granted by EPA under FFDCA, PIPs should not be field tested in a manner which will allow for pollen transfer to other sexually compatible crops or wild relatives. The adventitious presence of an unapproved pesticide in a food or feed product is no small matter and can lead to expensive testing of shipments of grain and other commodities as well as expense, penalties and embarrassment for the involved parties, despite their best intentions in field testing.

EPA is currently revising its regulations regarding PIPs and codifying the data requirements needed to obtain an experimental use permit and to achieve full registration under FIFRA. As new technologies (*e.g.*, RNAi) evolve, the Agency will need to update its regulations to maintain a science-based assessment process focusing on risk. As a scientist, you may be called upon to aid in this process through the FIFRA mandated science advisory panel or public comment. We hope you will participate!

WHY CAN'T WE ALL JUST GET ALONG? DIFFICULTIES IN PRIVATE-PUBLIC SECTOR COLLABORATIVE RELATIONSHIPS

Enno Krebbers

DuPont/Pioneer Crop Genetic Research
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It seemed like a simple idea at the beginning. Scientists at University X would collaborate with Company Y. The ideas were complementary and everyone got along so well. Of course one cannot predict exactly where the research will lead, so commercial terms (what are those?) were left to be determined later, when the value (what is that?) of the product of the research (do we really agree on what it is?) could be defined (how is that done?). And then it got really complicated, all kinds of non-scientists got involved, and suddenly we were not having fun anymore. This talk will give a company scientist's perspective on the differences between value, value sharing, and value capture. It will discuss why risk sharing means sharing the low end as well as the high end risk, and the role intellectual property plays in all this.

PROTEOMIC ANALYSIS OF DROUGHT RESISTANCE IN CRABAPPLE SEEDLINGS PRIMED BY THE XENOBIOTIC β-AMINOBUTYRIC ACID

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In a variety of annual crops and model plants, the xenobiotic DL-β-aminobutyric acid (BABA) has been shown to enhance disease resistance and increase salt, drought and thermotolerance. BABA does not activate stress genes directly, but sensitizes plants to respond more quickly and strongly to biotic and abiotic stresses. This process is referred to as chemical priming. Primed plants do not suffer from costly defense investments (such as inhibition of photosynthesis) since their defense arsenal is not activated before stress exposure. However, there are no reports on BABA-induced resistance in woody species. Additionally, the metabolic pathways through which BABA mediates both abiotic and biotic stress resistance are still being elucidated. In the present study, drought tolerance of four-week-old crabapple (*Malus pumila*) seedlings was significantly increased ($P \leq 0.05$) following a soil drench treatment with 500 µM BABA. On the tenth day after cessation of watering, the level of water loss in BABA-primed seedlings was 2-3 fold less than that of untreated plants, clearly indicating the ability of BABA to induce tolerance to drought stress in perennial plants. 2-D Difference in-Gel Electrophoresis (DiGE) was employed to characterize and compare differences in protein expression in leaf tissue sampled from control, BABA-primed and ABA-treated seedlings exposed to drought stress. A comparison of the different treatment combinations on the third and tenth day of dehydration revealed that 102 and 202 proteins, respectively, were differently expressed ($P<0.05$), in at least one condition. Among those, there were proteins that showed almost identical patterns of upregulation (57) or down regulation (34) in BABA and ABA treated seedling that supports the general concept suggesting that BABA-induced resistance in plants is achieved by potentiating ABA-regulated pathway. However, some differentially expressed classes of proteins were uniquely up-regulated (54) and down-regulated (38) only in BABA-primed plants, indicating that BABA may also mediate resistance via some ABA-independent pathways. MALDI-TOF MS/MS is being utilized to identify the proteins of interest. A quantitative analysis of the proteomes of control, ABA and BABA-treated tissue will be presented together with the discussion of possible mechanisms of BABA-mediated resistance in woody plants.

IDENTIFICATION AND CHARACTERIZATION OF ACD6-1 SUPRESSOR 6, A NOVEL DEFENSE GENE IN ARABIDOPSIS

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Pathogen infection activates complicated defense signaling networks in plants. It remains challenging to identify defense genes and understand the mechanisms of action of these genes. Accelerated Cell Death 6 (ACD6) is an important positive regulator of Arabidopsis defense that act through the key signaling pathway mediated by salicylic acid (SA). A gain of function mutant, *acd6-1*, has hallmarks of extremely small size, constitutive resistance, cell death, and accumulation of high levels of SA. In a comprehensive genetic analysis to study the regulation of *acd6-1* conferred phenotypes, we found that SA but not ET/JA mediated signaling is required for *acd6-1* conferred small size and other phenotypes and SA antagonizes ET/JA in *acd6-1*. We exploited the unique SA-dependent dwarfism of *acd6-1* in a large-scale of genetic screen to identify novel defense genes.

Among the genes identified from this screen were *SUP1* and *SUP6*. *sup1* is an allele of *SA INDUCTION-DEFICIENT 2* (*SID2*) which is involved in SA biosynthesis. *SUP6* encodes an uncharacterized metalloprotease. Two *sup6* mutant alleles suppressed *acd6-1* conferred phenotypes and led to enhanced disease susceptibility to *Pseudomonas syringae* infection. Therefore, we have validated the potential of this genetic screen in identifying novel defense genes. Further investigation of SUP6 function will advance our understanding of the mechanisms of plant defense.

PSEUDOMONAS SYRINGAE AND PLANTS: A RELATIONSHIP BUILT ON LETHAL INJECTIONS

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Plants are attacked by diverse microbial pathogens that share an ability to transfer "effector" proteins into host cells to suppress innate immune responses. *Pseudomonas syringae* pv. *tomato* DC3000, a pathogen of Arabidopsis and tomato, has become an important model for studying effector-mediated pathogenesis and plant defenses. DC3000 virulence is dependent on its ability to inject 28 effectors into host cells via the type III secretion system (T3SS) injectisome. The complete repertoire of DC3000 effectors and injector-related proteins was identified by genome-enabled bioinformatic/experimental approaches. Analysis of this repertoire revealed multiple redundancies in injectisome and effector proteins, which has necessitated the construction of bacterial polymutants lacking complete sets of redundant factors and the development of various gain-of-function assays that can be used with isolated effectors. One such assay involves a screen for effectors with deleterious effects when inducibly expressed in yeast. Seven of 27 effectors tested show such toxicity, presumably because they disrupt universal eukaryotic functions. To identify specific effector targets in tomato, effectors lacking toxicity in yeast were used as baits in a yeast two-hybrid screen for interactors in a defense-induced tomato cDNA library. The putative DC3000 effector-host interactome reveals potential novel targets involved in ubiquitination, transcription, and vesicle trafficking. Finally, our efforts to facilitate community exploration of the highly multifactorial interactions of DC3000 and plants as a systems biology problem will be discussed.

ESTIMATES OF SPATIAL GENETIC STRUCTURE WITHIN AND AMONG CLONES OF WILD LOWBUSH BLUEBERRY IN MAINE USING EST-PCR MOLECULAR MARKERS

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Using recently developed EST-PCR markers, several levels of spatial genetic structure of wild lowbush blueberry, *Vaccinium angustifolium* Ait., were investigated. Knowledge of this structure (or lack of it) is a prerequisite to testing hypotheses regarding the dramatic yield variation among

individuals or clones as a possible result of near-parental inbreeding depression. Lowbush blueberry is largely self-incompatible since selfing rates are very low relative to outcrossing rates. This is well known in the literature and has been shown here, too, by three years of field hand-crosses among genotyped individuals in two fields in Maine. Leading researchers in lowbush blueberry have conjectured that perhaps closely spaced groups of highly related clones, who likely receive most pollen from themselves and their nearest neighbors (because of near-neighbor honey bee pollination patterns), could be a viable explanation of these dramatic differences in yield. If this hypothesis is true, one would expect to find these two properties of the system: 1) that structure does exist and that there should be indications of positive spatial autocorrelation within fields, i.e. clumping of related individuals who exchange pollen, and 2) that clusters are closely enough related to incite a decrease in yield due to inbreeding depression. The work presented here focuses on the first of these. We have attempted to break this structure into several logical levels: 1) intraclonal, 2) among clones within a field and 3) among three fields separated by 18 km. Data will be presented that shows that clones show high but not complete intraclonal fidelity. Also, transects within fields show that there is no correlation between genetic and physical distance of clones. Several analyses are presented including AMOVA (Analysis of Molecular Variance), Principal Component Analysis and various methods of spatial autocorrelation which all indicate a 'trend' of isolation by distance only at longer distances between fields. In summary, our quantitative molecular evidence supports the characterization of the lowbush blueberry species as a 'phalanx' clonal form with an 'initial seedling recruitment' life history as opposed to 'repeated seedling recruitment'. Therefore, the structure that we currently see in the fields of these long-lived woody perennials could be the result of 13,000 years (last glaciation period in Maine) of long distance dispersal of seed (diploid gene flow) by birds and bears. This could have resulted in a random seed bank of individuals which then were allowed to colonize and take over fields that have been burned, cleared and harvested by mankind for several centuries. If true, these conclusions do not support the idea that proximal, highly related clones cause significant field level suppressions of yield by inbreeding depression. Subsequent and ongoing work in field hand pollinations are revealing that differences in selfing rate strongly predict general outcrossing yield and may be interacting with genetic relatedness in a complex dynamic.

CONFIRMING AND CHARACTERIZING MICRORNAS THROUGH EXPRESSION PROFILING

Jeanne Wilson

DuPont/Pioneer Crop Genetic Research

MicroRNAs (miRNAs) are a conserved class of 21nt small RNA molecules that exert profound impact on plant developmental and adaptive processes. By acting as sequence-specificity determinants for the RNA-induced silencing complex (RISC), these small RNAs direct the post-transcriptional regulation of specific genes. To better understand the expression patterns of known miRNAs as well as to screen candidate miRNAs, we constructed a microarray able to profile the expression of 230 known miRNAs and thousands of candidates in multiple plant species. Through profiling several tissues from Arabidopsis, soy, maize and rice, we demonstrated miRNA expression patterns that are broadly conserved throughout angiosperms. Using the behavior of known miRNAs as standards, we also used the array to evaluate the existence and expression of computationally predicted miRNAs and candidate small RNA from deep sequencing experiments. The results suggest that careful computational and molecular screening of small RNA pyrosequencing data is an effective, high-throughput method of miRNA discovery.

KEYNOTE ADDRESS

DYNAMIC ORGANIZATION OF THE LIVING PLANT CELL

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Labeling of plant compartments with fluorescent proteins expressed from nuclear or chloroplast transgenes has completely revised our notion of organelle morphology and intracellular dynamics. Mitochondria with diverse morphology can be observed fusing and splitting, Golgi bodies and vesicles are highly mobile, and plastids have unexpected features. The tubular extensions of chloroplasts that were repeatedly discovered, and then forgotten, prior to 1970, are now definitely established as features of chloroplasts and other plastids. These so-called "stromules" (stroma-filled tubules) are common on plastids in some types of cells but are only sporadic in mesophyll cells. Stromules extending from the main plastid body are frequently found in many different types of non-green plant cells. Stromules often appear to contact the plasma membrane and nuclear envelope, surround other organelles, and sometimes even pass through nuclei. Proteins move between plastids connected by stromules. While genetic evidence indicates chloroplast genomes do not usually traffic between plastids, RNAs and small DNAs may possibly be transmitted through stromules. Like chloroplasts, stromules exhibit motility mediated by the actin cytoskeleton. Current questions concern how stromules form and move and the nature of their roles within the plant cell. Stromules may function (1) to increase the envelope surface area to facilitate import and export (2) to transfer molecules between different plastids (3) to channel materials and signals to appropriate locations (4) to reduce diffusion distance between plastids and other subcellular compartments and (5) to facilitate autophagy during senescence.

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MKP1 ENHANCES CYTOKININ SENSITIVITY, PROMOTES EARLY FLOWERING AND FLORAL MERISTEM LONGEVITY IN TOBACCO PLANTS

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MAP kinase (MPK) signaling cascades are known to transmit both biotic and abiotic stress signals in plants. However, their roles in plant growth regulation are mostly unknown. We took advantage of a strong mammalian MPK phosphatase (MKP1) to investigate potential functions of MAPK cascades in plant growth and development. Unexpectedly, expression of MKP1 in cultured tobacco cells led to shoot differentiation from microcalli, indicating enhanced cytokinin sensitivity. Transgenic tobacco plants expressing MKP1 showed remarkable phenotypes, including early flowering, delayed leaf senescence, and increased longevity of floral meristems, which continued to produce new flowers even after seed set. Stem segments from MKP-1 plants were consistently able to produce shoots on medium without exogenous cytokinin, and these shoots also flowered precociously in culture. Flowers from most of the MKP1 transformed lines had protruding stigma and enhanced anthocyanin accumulation in petals, resembling flowers of tobacco plants that have reduced ethylene sensitivity due to the expression of a dominant mutant of ethylene receptor ETR1. All the phenotypes associated with MKP1 depended on the phosphatase activity of MKP1, since expression of a phosphatase-inactive mutant, MKPCS, did not result in the same phenotypes. Analysis of gene expression in MKP1 plants showed elevated expression of a type A response regulator, a pathogenesis-related protein (PR1-b), and an Flowering locus T gene (FT) gene while the expression level of ACC oxidase (ACO, the enzyme for the final step of ethylene biosynthesis), was decreased compared to that of the wild-type plants. These results suggest that MKP1 might inactivate stress-related MPK pathways, and as a result, increase the sensitivity to cytokinin. MAPK pathways may be a convergent point through which stress and growth signals interact in shaping the final outcome of plant development.

A ROLE FOR STRIGOLACTONES IN ARABIDOPSIS: A NEW PLANT HORMONE

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Parasitic weeds of the genera *Striga* and *Orobanche* are considered the most damaging agricultural agents in the developing world. In Africa alone *Striga* species have infested up to two-thirds of the arable land and are thought to cause tens of billions of dollars in lost crop yields. To ensure coordination with a host, parasitic plant seeds only germinate when they sense a group of related compounds, called strigolactones, which are released by the host root. Although this makes strigolactone synthesis and action a major target of biotechnology the parasitic lifestyle and the lack of molecular and genetic tools makes studies on these weeds problematic.

Although parasitic plants use strigolactones produced by the host as a germination stimulant, the first active strigolactone, Strigol was actually purified from a non-host cotton plant, which is not normally infected by Striga species. The ability of non-host plants to germinate parasitic plant seeds suggests strigolactones may exist ubiquitously in higher plants and have roles that are independent of host-parasite interactions.

Here we show using a combination of chemical and classical genetics that, as observed in parasitic plants, strigolactones can play an analogous role in seed germination in the model organism Arabidopsis. We identified mutants deficient in phytochromobilin synthesis that require strigolactones for good germination and show these mutants are inefficient at stimulating parasitic seed germination. A rice mutant deficient in phytochromobilin synthesis is also inefficient at stimulating *Striga* germination demonstrating that *Arabidopsis* may be useful in identifying genes for crop breeding programs against parasitic weeds. We are also able to show that many aspects of the phytochromobilin deficiency are rescued by strigolactone addition suggesting these compounds may play an important role in light and retrograde signaling in plants. It therefore appears that strigolactones not only function as signaling molecules between parasitic and host plants but also act endogenously like a hormone in germination and early seedling growth.

CYTOKININ SIGNALING AND STEM CELL SPECIFICATION

Bruno Mueller, Ildoo Hwang & **Jen Sheen**

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Cytokinins are key regulators of a large number of processes in plant responses to external stimuli and in plant development, which is highly plastic and adaptive, and remarkably resilient and self-perpetuating. The cytokinin signal transduction pathway involves a multistep two-component system, including hybrid histidine protein kinase sensors, phosphotransfer proteins, and regulators as transcription activators and repressors in a phosphorelay system. Each step is executed by components encoded by multigene families with overlapping as well as distinct roles. Recent findings have revealed that the cytokinin signal transduction pathway can be modulated by nutrients, stress, and other hormones, and is integrated into a signaling network in both shoots and roots. To investigate the control of two-component signaling at the molecular and cellular level *in planta*, we introduce a synthetic reporter to visualize universally cytokinin output in vivo. Unexpectedly, the first embryonic signal is detected in the hypophysis, the founder cell of the root stem-cell system. Its apical daughter cell, the precursor of the quiescent centre, maintains phosphorelay activity, whereas the basal daughter cell represses signaling output. Auxin activity levels, however, exhibit the inverse profile. We show that auxin antagonizes cytokinin output in the basal cell lineage by direct transcriptional activation of *ARR7* and *ARR15*, encoding feedback repressors of cytokinin signaling. Loss of ARR7 and ARR15 function or ectopic cytokinin signaling in the basal cell during early embryogenesis results in a defective root stem-cell system. These results provide a molecular model of transient and antagonistic interaction between auxin and cytokinin critical for specifying the first root stem-cell niche.

THE SPLICING FACTOR GENE *SR45* FUNCTIONS THROUGH BOTH SPLICING ISOFORMS IN *ARABIDOPSIS THALIANA*

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Regulation of pre-mRNA splicing generates functional protein diversity and is evolutionary conserved. The serine-arginine-rich (SR) proteins constitute a duplicated and conserved family of pre-mRNA splicing factors. Prior work has shown that a mutation in one of these, *sr45-1*, has pleiotropic phenotypes, suggesting that SR45 regulates genes that are involved in different developmental processes. Like most SR protein genes, *SR45* itself is alternatively spliced. However, the biological implication of alternative splicing in the *SR45* gene remains unknown.

SR45 is ubiquitous in plants. Its relationship to SR proteins in animals is unclear. However, SR45 may be orthologous to the animal protein RNPS1, which is a core component of the exon junction complex (EJC), a conserved multiprotein complex that mediates mRNA export and nonsense-mediated mRNA decay. In SR45, alternatively spliced isoform 1 has an insertion of 7 amino acids (TSPQKTG) relative to isoform 2. We individually overexpressed both *SR45* isoforms as GFP fusions in *sr45-1* mutants. We found that overexpression of isoform 1 rescued the flower development phenotype of *sr45-1* mutants, while overexpression of isoform 2 complemented the root growth of *sr45-1* mutants. GFP fluorescence was observed in nucleoplasm in transgenic plants overexpressing either isoforms. Transgenes producing isoform 1 with the substitutions 219S to A did not rescue the late flowering phenotype of the mutants. This result suggests that this serine may be crucial for isoform 1 function. Serine^{219} is predicted phosphorylation site, so isoform 1 activity in flowering may require phosphorylation of Serine^{219}. In conclusion, our study provides evidence that the two alternatively spliced isoforms of *SR45* have distinct biological functions. This would be an example of alternatively spliced SR protein isoforms having distinct functions beyond homeostatic or cross-regulatory effects on SR proteins.

BRASSINOSTEROID SIGNAL TRANSDUCTION AND REGULATION OF PLANT DEVELOPMENT

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Brassinosteroids (BRs) are essential hormones that regulate a wide range of processes in plants. BR deficiency or insensitivity causes multiple growth defects, including dwarfism, male sterility, delayed flowering, and light-grown morphology in the dark. Molecular genetic studies in Arabidopsis have identified a number of BR signal transduction components, yet the pathway remains Incomplete and the links to specific cellular and developmental processes remain unclear. BRs bind to the BRI1 receptor kinase at the cell surface and regulate the BZR1 and BZR2 (also named BES1) transcription factors through a signaling cascade that include the BIN2 kinase and the BSU1 phosphatase. When BR levels are low, BIN2 kinase phosphorylates BZR1 and BZR2/BES1 to inhibit their DNA binding and promotes their cytoplasmic retention by the 14-3-3

proteins. BR induces dephosphorylation of BZR1 and BZR2 by inhibiting BIN2 and/or activating BSU1. Using proteomic studies we have identified a class of BR signaling kinases (BSKs) that mediate BR signal transduction from the BRI1 receptor kinase. Using chromatin immunoprecipitation-microarray (ChIP-chip), we identified over 3000 BZR1-target genes, which reveal the molecular links between BR and various hormonal and developmental pathways. Functional studies of BZR1-target genes are establishing a link between BR and lateral organ development. A complete signaling pathway from BR perception to cellular and developmental responses is emerging.

COMPARATIVE AND EVOLUTIONARY GENOMICS OF SOYBEAN (GLYCINE MAX) AND COMMON BEAN (PHASEOLUS VULGARIS)

Scott Jackson

Purdue University

Soybean (*Glycine max*) is an economically important crop for food and fuel. Its genome is large (~1,100 MBP/haploid genome) and complicated by several rounds of polyploidization and/or segmental duplications followed by structural rearrangements. A preliminary genome sequence of soybean was released in January of 2008 by the Joint Genome Institute of the Department of Energy, USA. Genome wide analysis of duplications within soybean confirm that the genome is highly duplicated with many genes present in at least four copies. Moreover, extensive structural rearrangements are seen where duplications are nested within other duplications. Due to the complex nature of the soybean genome, we have developed genomic resources for common bean (*Phaseolus vulgaris*) which has a smaller genome (~600 Mbp/haploid genome) and appears to be diploid. A BAC-based physical map with associated end sequences has been developed and we have aligned the physical map to the soybean genome sequence. We have also sequenced a targeted region of ~1Mbp from *Phaseolus* and have sequenced two orthologous regions from soybean. The *Phaseolus* sequence is highly conserved with both soybean orthologs and is useful for determining ancestral gene order and putative rearrangements in either or both of the soybean copies. This work will help in the future sequencing of *Phaseolus* as a diploid model for Phaseoloid legumes and for common bean improvement and will lead to a better understanding of the soybean genome for developing better yielding and adapted soybeans.

DuPONT BIOFUELS

William D. Hitz

DuPont Central Research and Development, Wilmington, Delaware

Transportation fuels are not the largest use of fossil fuel energy but the category is the fastest growing and is more difficult to substitute into than the fixed energy conversion uses such as power plants. Fuels that substitute into the existing, petroleum derived fuel stream need to do so with minimal disruption to the large infra structure that feed the internal combustion engine. This makes conversion of biomass to alcohols an attractive target to substitute for increasing amounts of petroleum. If this substitution is to be a significant part of transportation fuel it will require an extremely large investment in process facilities and commitment of biomass to fuel use.

Process development for conversion of biomass to fuel alcohol needs to take into account many factors from appropriate feedstock supply through investment size, environmental impact and energy yield. I will describe the DuPont Integrated Corn BioRefinery approach and how it tries to do this and how that relates to feedstock quality and supply.

AN INVESTIGATION OF HETERODERA GLYCINES (SOYBEAN CYST NEMATODE) GENE EXPRESSION AND A CANDIDATE GENE APPROACH TO STUDY ITS DEVELOPMENT

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Parasitic nematodes are a major agricultural problem that is poorly understood. The most prominent of these interactions is *G. max* infection by *H. glycines* because it accounts for an estimated $460 to $818 million in production losses annually in the U.S. A microarray analysis of ~7500 probe sets on the Affymetrix® soybean Genechip® was used to study soybean cyst nematode gene expression during infection. In a companion analysis, candidate nematode genes were used in a series of plant transformation experiments with the aim of understanding their role during nematode infection. This was accomplished using a panel of RNAi vectors developed specifically for soybean transformation and nematode investigation.

01 MONSANTO SEQUENCE 3 CONTIG ANNOTATION PROJECT AT USDA

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Background: One of the objectives of of the Soybean Genomics and Improvement Laboratory is to develop a transgenic approach to broaden resistance of soybean, *Glycine max,* to the soybean cyst nematode (SCN), *Heterodera glycine,* that causes an estimated loss of one billion dollars in the US annually. The goal of this project carried out by UMUC Capstone Course students in Bioinformatics is to annotate the first 100,000 base pairs of *Glycine max*, Monsanto sequence 3, which is believed to carry one of the genes for resistance to SCN.

Methods: Monsanto Sequence Part 3 from Patent WO0151627 was retrieved with the accession number AX196296 from NCBI. The sequence was submitted for analysis to three gene-finding programs, GenScan, GeneMark and FGenesh selecting as training sets, *Arabidopsis thaliana* for both GenScan and GeneMark and *Medicago truncatula* for FGenesh. Potential genes and their locations were then identified based on coding regions predicted by all the programs. The promoters, poly-A-tails, and other signals such as TATA boxes, CAAT boxes, Transctiption Start Site (TSS) were used to corroborate our findings. Software tools such as BLAST, BLASTx, BLASTn, BLAST2Seq, and the more sophisticated tool to view the annotations using a graphical interface, Artemis, were also used. Databases such as TIGR, ExPASy, and Pfam were used to further verify our findings.

Results: A total of 17 tentative gene sequences were identified from the gene finding tools, though not all of them predicted all the genes, and not all genes were predicted at the same location. In an effort to verify the results, the sequences of predicted genes were translated to protein and searched against the GenBank database of proteins through BLASTx. 13 of the 17 tentative genes had a similarity to existing proteins in GenBank. The protein sequences from the Monsanto sequence and GenBank sequences were compared using BLAST2Seq, and 15 regions of similarity were obtained.

Conclusion: A summary of the conclusion of annotating the first 100,000 bp of Monsanto Sequence 3:

Gene 1- No conclusion made / may or may not be a gene - more analysis needed in future
Gene 2- Heavy Metal Transport Protein *(Medicago truncatula-* ABE84888.1)
Gene 3- Conserved Hypothetical Protein *(Medicago truncatula-* ABE84887.1)
Gene 4- No solid database result / may or may not be a gene – more analysis needed in future
Gene 5- Prephenate Dehydrogenase Protein *(Medicago truncatula-* ABE84886.1)
Gene 6- Trimeric Lipase A like Protein *(Medicago truncatula-* ABE84885.1)
Gene 7- Single Hybrid Motif *(Medicago truncatula-* ABE84884.1)
Gene 8- Conserved Hypothetical Protein *(Medicago truncatula-* ABE84883.1)
Gene 9- No solid database result / may or may not be a gene – more analysis needed in future
Gene 10- Receptor like Kinase RHG1 (*Glycine max* – AAM44273.1)
Gene 11- Laccase Diphenol Oxidase (*Glycine max* – AAM54731.1)
Gene 12- Sodium Hydrogen Antiporter Protein *(Medicago truncatula-* ABE84874.1)
Gene 13- No solid database result / may or may not be a gene – more analysis needed in future
Genes 14,15,16- ATP Binding Helicase *(Medicago truncatula-* ABE84873.1)
Gene 17- No solid database result / may or may not be a gene – more analysis needed in future

02 REGULATORY INTERACTION BETWEEN AN INOSITOL POLYPHOSPHATE 5-PHOSPHATASE AND SNRK1.1 (AKIN10) LINKS INOSITOL, SUGAR AND STRESS SIGNALING

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In plants, inositol signaling pathways have been associated with several stress, developmental and physiological processes, but the regulation of these pathways is largely unknown. In our efforts to better understand inositol signaling pathways in plants, we have found that the WD40 repeat region of an inositol polyphosphate 5-phosphatase, (5PTase13, At1g05630), interacts with the sucrose nonfermenting-1-related protein kinase (SnRK1.1) in the yeast two-hybrid system and *in vitro.* Plant SnRK1 protein kinases (also known as AKIN10 /11) have been described as central integrators of sugar, metabolic, stress and developmental signals. By using mutants, defective in 5PTase13, we show that 5PTase13 can act as a regulator of SnRK1 activity, and that regulation differs with different nutrient availability. Specifically, we show that under low nutrient or stress conditions 5PTase13 acts a positive regulator of SnRK1 activity. In contrast, under severe starvation conditions, 5PTase13 acts as a negative regulator of SnRK1 activity. To delineate the regulatory interaction that occurs between 5PTase13 and SnRK1.1, we used a cell free degradation assay and found that 5PTase13 is required to reduce the amount of SnRK1 targeted for proteasomal destruction under low nutrient conditions. This regulation most likely involves a 5PTase13:SnRK1.1 interaction within the nucleus, as a 5PTase13:green fluorescent protein fusion was localized to the nucleus. We also show that a loss-of-function in 5PTase13 leads to nutrient level-dependent reduction of root growth, along with ABA and sugar-insensitivity. *5ptase13* mutants accumulate less inositol (1,4,5)P_3 in response to sugar stress and have alterations in ABA-regulated gene expression, both of which are consistent with the known role of $InsP_3$ in ABA-mediated signaling. We propose that by forming a protein complex with SnRK1.1 protein kinase, 5PTase13 plays a regulatory role linking inositol, sugar and stress signaling.

03 *PHAKOPSORA PACHYRHIZI* GENE EXPRESSION DURING INFECTION IN SOYBEAN

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Soybean is one of the top five agricultural products in the United States with a value exceeding $19.7 billion in 2006. Protection of soybean from present and new exotic pathogens is very important for soybean production. Asian Soybean Rust (ASR), *Phakopsora pachyrhizi*, is an exotic pathogen first observed in the United States in 2004 in Louisiana. Since then, ASR has gradually moved north into the US major soybean production areas. This pathogen causes yield losses due to premature defoliation, fewer seeds per pod and decreased number of filled pods per plant. From this perspective we identified genes from Asian Soybean Rust that might be involved in different steps of infection, development and reproduction. Also we wish to identify soybean genes that may be useful to control ASR. Thus, we constructed and analyzed cDNA libraries to identify candidate genes. The libraries were constructed from RNA isolated from uredinia formed by the ASR on the under side of leaves and from palisade layer cells. Uredinia and palisade cells were isolated by laser capture microdissection. A portion of the uredinia library was sequenced, contigs were

formed, and blast searches were conducted to determine the identity of the genes. RT-PCR was used to confirm expression of six rust genes identified in the library and ten more genes frequently expressed in plant-rust interaction. In the future, target pathogen genes will be studied to determine if they can be used to control ASR in soybean.

04 COMPARATIVE GENOME ANALYSES OF *MONILIOPHTHORA PERNICIOSA* AND *MONILIOPHTHORA RORERI*: TWO CLOSELY RELATED PHYTOPATHOGENIC BASIDIOMYCETES THAT CAUSE DISTINCTLY DIFFERENT DISEASES OF *THEOBROMA CACAO*

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Theobroma cacao (cacao), the source of chocolate, is a tropical understory tree. Fungal diseases such as Witches' Broom Disease (WBD) and Frosty Pod Rot Disease (FPRD) of cacao have devastated cacao production in much of the Western Hemisphere and are threats to the main cacao producing regions in Africa and Southeast Asia. WBD is caused by the fungus *Moniliophthora perniciosa* (formerly known as *Crinipellis perniciosa*), and FPRD is caused by *Moniliophthora roreri*. Utilizing a combination of sequencing systems we have completed detailed draft genomes of *M. perniciosa* and *M. roreri*. A total of 325 Mbp (approx. 7X coverage) of *M. perniciosa* and 844 Mbp (approx. 16X coverage) have been sequenced. The genome sizes predicted from these sequences are 46 Mbp for *M. perniciosa* and 52 Mbp for *M. roreri*. A comparison at the nucleotide level shows that 70% of the sequences have 80% identity. This comparison gives similar results at the AA level with 70% of the sequences having 75% identity. The comparative analyses of these genomes have identified several pathogenicity genes in common and will be important for understand key physiological, morphological and pathogenic differences between these two closely related fungal pathogens.

05 DISSECTING GENETIC STRUCTURE IN FARMER SELECTIONS OF *THEOBROMA CACAO* IN THE PERUVIAN AMAZON: IMPLICATIONS FOR ON-FARM CONSERVATION AND REHABILITATION

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Natural evolution and human intervention have both contributed to on-farm diversity of crop species. Knowledge of genetic structure in farmer selections is essential to identify genetic entities that are priorities for conservation in farmers' fields. Using a capillary genotyping system with 15

microsatellite loci, we analyzed population structure in 220 farmer selections of cacao from the Peruvian Amazon. A high level of genetic diversity was observed in these farmer selections. The expected heterozygosity was 0.73 and the average number of alleles was 8.8, which are comparable to those of the cacao populations found in the Huallaga and Ucayali valleys of Peru. Bayesian ancestry inference revealed a multi-cluster admixture in the 220 farmer selections. A significant ancestry contribution (46.8%) was found from a local population in the Huallaga valley of Peru. The remaining ancestry contribution (53.2%) was explained by the introduced international clones. Among these introduced international clones, parentage analysis identified 16 clones as probable female parents and 11 clones as probable male parents for these farmer selections, including both Trinitario and Forastero cacao. The result suggests that in spite of the large scale deployment of introduced hybrid mixtures over the years, there is still a significant amount of native germplasm in farmers' fields in the Peruvian Amazon. The wide range of genetic variability resultant from hybrid mixtures in these cacao farms provided a great opportunity for participatory selection of superior clones. The present study is the first research to dissect on-farm cacao genetic diversity in Peru. The resultant information is useful for assisting rational deployment of farmers' elite clones in the cacao rehabilitation programs, as well as for future genetic improvement of cacao.

06 FUNCTIONAL CHARACTERIZATION OF THE HOMOLOGS OF THE BROAD-SPECTRUM DISEASE RESISTANCE GENE *RPW8* IN ARABIDOPSIS

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The *RPW8* gene isolated from *Arabidopsis thaliana* confers broad-spectrum disease resistance to *Erysiphe* spp. that cause powdery mildew disease on many plant species. Despite having an atypical R protein structure, RPW8 activates a conserved salicylic acid (SA)-dependent defense pathway leading to hypersensitive cell death in response to powdery mildew challenge. All of the tested Arabidopsis accessions to date contain three closely linked homologs of *RPW8* named *HR1*, *HR2*, and *HR3* at the same locus that do not seem to contribute to powdery mildew resistance. Recent evolutionary analyses revealed that *RPW8* most likely originated from an *HR3*-like progenitor gene by gene duplication and functional diversification. To understand how the resistance function of *RPW8* originated, we have recently conducted genetic analyses and found that 1) overexpression of *HR1*, *HR2* or *HR3* individually in susceptible accession Col-0 resulted in enhanced resistance to powdery mildew; 2) knocking out these genes individually appeared to cause enhanced susceptibility; and 3) unexpectedly silencing these three genes simultaneously results in enhanced resistance to the pathogen and some developmental phenotypes that resemble those caused by overexpression of *14-3-3* lambda, whose product interacts with RPW8 in the yeast-two-hybrid. Our genetic data support the hypothesis that these *RPW8* homologs, particularly *HR3*, may play an important role in basal resistance via the conserved SA pathway against powdery mildew and perhaps other pathogens and suggest that there may be complicated genetic interaction between *HR1*, *HR2*, *HR3* and other defense components for regulation of plant basal resistance.

07 THE ROLE OF A PUTATIVE INOSITOL PHOSPHORYLCERAMIDE SYNTHASE IN PLANT PROGRAMMED CELL DEATH ASSOCIATED WITH DEFENSE

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The Arabidopsis gene *RPW8* confers broad-spectrum disease resistance to an important, polyphagous, biotrophic fungal pathogen, commonly known as powdery mildew. Recent studies suggest that *RPW8* may stimulate a highly conserved plant innate immunity pathway that is connected with programmed cell death (PCD; also known as hypersensitive response) and activation of basal resistance. A genetic screen using *RPW8*-mediated cell death as a reporter has proven to be effective in identifying novel components involved in regulation of plant innate immunity. Over 5 groups of *erh* (*enhancer of RPW8-HR-like cell death*) mutants have been identified in *RPW8* background by T-DNA tagging and the corresponding mutated genes may be involved in negative regulation of a (*RPW8*) cell death pathway associated with disease resistance. Initial characterization of *erh1* indicated that loss-of-function of *At2g37940* caused by a T-DNA insertion is responsible for the enhanced HR-like cell death phenotype. *ERH1* (*At2g37940*) encodes a highly conserved plant protein that shows homology to the mammalian sphingomyelin synthase (SMS) which converts ceramide to sphingomyelin. Therefore, it is likely that loss-of-function of *ERH1* may lead to accumulation of intracellular ceramide and cause cell death especially in *RPW8* background. This finding corroborates with the previous finding that loss-of-function mutation in *ACD5* encoding a ceramide kinase leads to ceramide accumulation and developmentally regulated cell death.

08 dbEstUMD: BIOINFORMATICS TOOL AUTOMATES SUBMISSION OF EXPRESSED SEQUENCE TAGS TO NCBI'S DBEST DATABASE

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The dbEST database is one of many valuable databases of genomic and proteomic data in digital form under the umbrella of the GenBank and NCBI. The process of preparing expressed sequence tags (EST) and the associated annotations for inclusion in the dbEST database is a tedious process, even with the assistance or *ad hoc* programs. dbEstUMD was written to provide a publically available and complete web based solution for EST sequence submissions. The goal of the dbEstUMD bioinformatics tool is to provide an easy to use web application which collects, organizes and submits to NCBI the EST sequence data and associated information according to the standards provided by NCBI. The tool will be made available to the public in September 2008. Subsequent releases may be forthcoming to enhance and refine the tool beyond its initial basic dbEST submission processes.

09 NEW MUTANTS WITHIN THE ETHYLENE SIGNALING PATHWAY IN *ARABIDOPSIS*

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The plant hormone ethylene is an important regulator of plant growth and development, including senescence, abscission, fruit ripening, and responses to biotic and abiotic stresses. We have performed a mutagenesis screen to isolate new players in the ethylene signaling pathway. Ethylene is perceived by a family of five receptors in *Arabidopsis thaliana,* which negatively regulate the response to the hormone. The ethylene-insensitive *etr1-2* is dominant gain-of-function allele within the ETR1 ethylene receptor. We mutagenized *etr1-2* seeds with the goal of identifying suppressors of this mutation. The screen was carried out by searching for mutants that exhibited the triple response, a phenotype characteristic of wild-type etiolated seedlings grown in the presence of ethylene. Here, we present two mutants isolated from the *etr1-2* suppressor screen. The first mutant, *etr1-10*, is an intragenic mutation within *ETR1*, a unique missense mutation that apparently eliminates ETR1-2 signaling. The second mutant, *rte3* (*reversion to ethylene-sensitivity3*), is extragenic and is located within a previously uncharacterized protein. There is no known function for *RTE3* in *Arabidopsis*. Current work focuses on the characterization of the *rte3* and *etr1-10* mutants.

10 MOLECULAR CHARACTERIZATION OF *StMyb1* AND *StRD22* GENES FROM POTATO PLANT

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The drought tolerance is one of the major targets for the future agriculture. Biotechnology has the potential to improve the drought tolerance of crops by transgenic plant technology. The limiting factors for developing this technology are the isolation of genes those active function in drought tolerance and the precise understanding of the molecular process of drought tolerance. In order to analyze the response of plants to drought stress at the molecular level, a number of cDNA clones which are related to drought tolerance were isolated from *Solanum tuberosum* L. Among these, we focused two genes, *StMyb1* and *StRD22*. The expression of *StMyb1* and *StRD22* was induced by exposure to salt, ABA, and mannitol. The StMyb1 protein was localized to the nucleus of onion epidermis cells suggesting it is potential transcriptional factor. For biofunctional analysis of *StMyb1* and *StRD22*, a pBI121 vector containing *StMyb1* and *StRD22* gene under the control *of CaMV 35S* and *RD29A* promoter, respectively, was constructed for plant transformation. The resulting *StMyb1* and *StRD22* transgenic plants showed increased tolerances on salt and drought stresses. In addition, by microarray analysis using TIGR 10K potato chip and *StMyb1* transgenic plants, we identified a number of downstream genes of *StMyb1* including a number of transcription factors and stress inducible genes (CG3134).

11 SOUND STIMULATES GENE EXPRESSION AND PATHOGENS CONTROL

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We investigated whether sound could alter gene expression in plants. Using a sound-treated subtractive library, a set of sound-responsive rice genes was demonstrated through mRNA expression analyses. The sound stimuli were complex musical sounds and single frequencies. Under both light and dark conditions, sound up-regulated the expression of *rbcS* (ribulose small subunit), *ald* (fructose-1,6-bisphosphate aldolase), and DNA-J-like gene. These are also light-responsive genes and these results suggest that sound could represent an alternative to light as a gene regulator. The expression of *ald* mRNA increased significantly with treatment at 125 and 250 Hz, whereas the expression levels decreased significantly with treatment at 50Hz, indicating a frequency-specific response. To investigate whether the *ald* promoter responds to sound, we generated transgenic rice plants harboring a chimeric gene comprising a fusion of the *ald* promoter and *GUS* reporter gene. In three independent transgenic lines treated with 50 or 250 Hz for 4 h, the sound-responsive *GUS* mRNA expression pattern under the control of the *ald* promoter closely correlated with the expression pattern of the *ald* gene, suggesting that the 1,506-bp *ald* promoter is sound-responsive. Therefore, we propose that, in transgenic plants, specific frequencies of sound could be used to regulate the expression of any gene fused to the *ald* promoter.
We also examined whether frequency-specific sound signal can control pathogens by the growth inhibition of fungi and bacteria. When a single specific sound frequency was applied to *Botrytis cinerea* growing on solid medium, mycelial growth was inhibited and spore formation was reduced. The organs were also deformed. In addition, the mycelia treated with sound showed decreased spore formation, indicating that the effect continued into the next generation. When cucumber leaves that were infected with *B. cinerea* were treated with the specific sound frequency, the symptomatic area was decreased significantly as compared to the control group. This demonstrates that a specific sound frequency might be used, not only to increase plant growth, but also to rapidly control disease and counter severe environmental stress.

12 USING AN APPLE (*MALUS*) MICROARRAY FOR EXPRESSION ANALYSIS OF RESPONSES TO COMPATIBLE AND INCOMPATIBLE PATHOGENS

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Fire blight is a devastating disease of apple (*Malus* x *domestica*) caused by the bacterial pathogen *Erwinia amylovora* (Ea). Ea enters the plant through blossom nectaries or wounds, multiplies in the apoplast and spreads through the plant via vascular tissues. When infiltrated into host leaves, Ea induces lipid peroxidation, electrolyte leakage, and antioxidant enzyme changes, similar in intensity and kinetics to the oxidative burst observed during the development of a hypersensitive response

(HR) following inoculation with an incompatible rather than a compatible pathogen (Venisse *et al.* 2001. Pl. Physiol. 125:2164-72). *Pseudomonas syringae* pv. *syringae* strain B86-6 (Pss) is a broad host range bacterial pathogen that is incapable of producing disease in apple but produces an HR response upon infiltration into apple leaves (incompatible pathogen). The objective of this study was to compare the defense response of *Malus* to challenges by Ea and Pss using microarray analysis to gain insight into how Ea overcomes defense reactions associated with HR and establishes systemic infection. To this end, we used a 40,000 feature, two-channel, printed *Malus* microarray that contains 548 control probes and 39,412 long-oligonucleotide (70-mer) probes designed to non-redundant *Malus* EST contigs (unigenes) obtained from different tissues, genotypes, developmental stages and stress conditions. Leaf tissues were harvested from apple shoots of fire blight susceptible 'Malling 26' rootstock inoculated with either phosphate buffer (Mock), virulent Ea strain Ea273, or Pss strain B86-6 at 6 h post-inoculation. RNA was isolated from tissues, and labeled with Alexa Dye 555 and 647. Several protocols were tested to optimize transcript labeling and microarray hybridization. A loop design was used to compare expression profiles of Mock vs. Ea, Mock vs. Pss, and Ea vs. Pss challenged tissues. Differentially expressed genes were identified using two interconnected mixed linear models for normalization of array data and determination of gene effects (Wolfinger *et al.* 2001 J. Comput. Biol. 8:625-37). A total of 1541 genes were differentially expressed in Mock vs. Ea, 995 in Mock vs. Pss, and 726 in Ea vs. Pss comparisons. Interestingly, a high number of differentially expressed genes (450) were common in both Mock vs. Ea and Mock vs. Pss comparisons, suggesting commonalities between both interactions, while 726 differentially expressed genes in the Ea vs. Pss comparison suggest there are also specific responses to both compatible and incompatible interactions. Bioinformatic analysis of differentially regulated genes is currently in progress to identify gene families and/or enzymatic pathways that are either common or specific to the compatible and incompatible host-pathogen responses in *Malus*.

13 TRANSGENIC EXPRESSION OF THE *ERWINIA AMYLOVORA* (FIRE BLIGHT) EFFECTOR PROTEIN EOP1 SUPRESSES HOST BASAL DEFENSE MECHANISMS IN MALUS (APPLE)

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Erwinia amylovora (*Ea*) is the causative agent of fire blight, a devastating disease of apple and pear. Like many other plant and animal bacterial pathogens *Ea* utilizes a type three secretion system (TTSS) to deliver effector proteins into plant host cells. Once inside the host cell, effector proteins are thought to function as inducers and/or suppressors of host defense responses; however, the exact mechanisms by which *Ea* effectors regulate these responses are not clearly defined. Several *Ea* effectors have been identified based on their sequence similarity to both plant and animal bacterial pathogen effectors. Eop1 (*Erwinia* outer protein 1) has sequence similarity to the animal pathogen *Yersinia_pseudotuberculosis* effector YopJ. To investigate the role of Eop1, M.26 apple was transgenically engineered to express the *Ea* effector protein Eop1 under the control of an inducible promoter. The Eop1 effector protein was directionally cloned from *E. amylovora* strain Ea273 into a Gateway™ compatible entry vector using gene specific primers that were modified to incorporate a Kozak sequence in the 5' end of the effector gene to facilitate proper translation in a eukaryotic system and a 6His tag at the 3' end for protein detection. Eop1 was subsequently cloned through Gateway technology into a binary vector, pBinPlusARS.XVE. This binary vector incorporates the regulatory elements of the estradiol-induced XVE gene expression system developed by Zuo *et al.* (2000) and was used in *Agrobacterium*-mediated

transformation of apple. Transgenic apple lines were confirmed through PCR analysis with effector specific primers and evaluated for *Agrobacteria* contamination with *virG* specific primers. Inducible expression of *eop1* in the presence of 25 uM estradiol was confirmed by RT-PCR. Callose deposition is a cellular marker of host basal defense, also known as innate immunity. When non-induced and induced leaf tissue of T98, a non-leaky Eop1 apple transgenic, was challenged with an *Ea* TTSS$^-$ mutant a significant reduction in callose deposition was observed in induced tissue, indicating that transgenic expression of Eop1 suppresses host basal defense mechanisms. In the future, these transgenic lines will enable us to continue investigating the role of Eop1 in regulating host defense mechanisms, as well as, determining its functionality when expressed *in planta* and its effect on host gene expression.

14 HOW DO PLANTS WITH ALTERED VITAMIN C COPE WITH STRESS?

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Myo-inositol (Ins) is an alternate precursor for Vitamin C synthesis, and it is used as a backbone of inositol trisphosphate (Ins(1,4,5)P_3) and phosphatidylinositol phosphate signaling molecules. Oxidation of Ins by the enzyme *myo*-inositol oxygenase (MIOX) produces D-glucuronic acid, which is further altered to produce Vitamin C. The Arabidopsis genome contains four genes predicted to encode MIOX enzymes. It was shown that plants ectopically expressing the MIOX4 gene from Arabidopsis (MIOX4$^+$) produced higher levels of Vitamin C. We are interested in whether the ectopic expression of the MIOX4 gene results in Ins signaling alterations, an important consideration for future Vitamin C genetic engineering efforts. We are also examining the loss-of-function mutants for phenotypes and signaling alterations. We have observed changes in flowering time and root length related to nutrient levels in the *miox2*$^-$ and *miox4*$^-$ mutants. We are measuring *myo*-inositol levels and other metabolites of the MIOX4$^+$ and *miox*$^-$ plants by GC analysis to determine if levels are altered. In the MIOX4$^+$ plants, we found a significant decrease in Ins and Ins(1,4,5)P_3 in seedlings. The decrease in Ins and Ins(1,4,5)P_3 was accompanied by insensitivity to ABA, a plant drought hormone that uses Ins(1,4,5)P_3 as a second messenger, while the miox2$^-$ mutants have sensitivity to ABA. This result suggests that MIOX expression affects a plant's ability to synthesize Ins(1,4,5)P_3 in response to ABA, and indicates that MIOX expression impacts Ins signaling as well as metabolism. Our results help us understand how MIOX function impacts *myo*-inositol signaling and metabolism and thus a plant's response to external stresses.

15 A FUNCTIONAL ANALYSIS OF THE PHD-FINGER DOMAIN CONTAINING PROTEINS, ING1 AND ING2, IN ARABIDOPSIS

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The Plant HomeoDomain (PHD) zinc finger was first identified in plants, though its presence is nearly ubiquitous in eukaryotes. It consists of a conserved zinc-binding Cys4-His-Cys3 motif. Recent reports have determined that conserved residues within some PHD fingers allow binding to trimethylated lysine 4 of histone H3 (H3K4me3), an important chromatin modification associated with transcriptional activity. In addition, some PHD fingers have been shown to bind to specific

phosphatidylinositol phosphates (PtdInsPs), which may be important for nuclear membrane localization. We have undertaken a bioinformatic analysis of the PHD finger in plants, specifically *Arabidopsis thaliana*. From this analysis we have determined that *Arabidopsis* has approximately 73 separate PHD finger domains that are contained in 60 different proteins. Most of these proteins also contained a domain annotated to function in chromatin remodeling, suggesting a major role for PHD containing proteins in this process. To begin to understand the role of the PHD domain in chromatin remodeling and inositol based signaling within Arabidopsis, we have identified and characterized an *Arabidopsis* loss-of-function mutant and a gain-of-function transgenic in the plant orthologs of the human tumor suppressor, INhibitor of Growth (ING). From these data we hypothesize that ING1 and ING2 serve a critical role and Arabidopsis growth and development.

16 ECTOPIC EXPRESSION OF POPLAR *FLOWERING LOCUS T1* GENE INDUCED EARLY FLOWERING AND NORMAL FRUIT DEVELOPMENT IN PLUM (*Prunus domestica* L.)

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Prunus is the most horticulturally valuable genus in the family Rosaceae. Members of this genus are cultivated for their fruits (peaches, plums, apricots, nectarines, cherries), nuts (almond) or for their ornamental flowers (flowering cherries). The importance of *Prunus* as crop species that provides nutrition and quality of life has led to world-wide efforts in gene identification including genome sequencing (http://www.bioinfo.wsu.edu/gdr/). Besides conventional breeding, application of molecular genetic technology such as structural and functional genomics and genetic engineering (GE) can be used to improve *Prunus* species. However, long juvenile period of up to 8 years is severe impediment in the analyses of gene function in both conventionally bred and transgenic *Prunus* fruit trees.

In order to develop early flowering *Prunus*, we used plum (*Prunus domestica)* as a model plant because efficient production of transgenic plums can be routinely achieved in our laboratory. To induce early flowering in plum, we used the poplar (*Populus trichocarpa*) *Flowering Locus T1* (*PtFT1*) gene and overexpression of this gene was shown to induce early flowering in poplar (Bohlenius et al. 2006). Ectopic expression of *PtFT1*under the control of the *35S* promoter induced early flowering in plum. Some transgenic plum plantlets produced cluster of flower buds and fully developed flowers in vitro. These plants when transferred to soil and grown in pots in the growth chamber also flowered. Normal flowering and fruiting were observed only when the transgenic plants were grown in the greenhouse. Pot grown transgenic plums in the greenhouse flowered within 2 to 4 months after planting and normal fruits developed and ripened 5 months after fruit set. Since plum is self-incompatible, pollen from different genotype were used to pollinate the flowers in transgenic plum plants. Under orchard conditions, plums normally flower and fruit only after 3-5 years. About five percent of the greenhouse grown transgenic plum plants produced flowers. Two plants set fruits and the fruits ripened normally. Flowers appeared as terminal panicles of 3 to 5 flowers or 1 to 3 flowers in leaf axils. Some leaf axils bore mixture of both vegetative and flower buds. Investigations of the differential expression of the *35S:: PtFT1* gene are underway.

17 FUNCTIONAL ANALYSIS OF NEMATODE-RESPONSIVE *PR* GENES IN SOYBEAN PLANTS

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PR (pathogenesis-related) genes are well known as plant defense-related downstream functional genes. It was reported that some of PR genes function not only in biotic stress but also in abiotic stress resistance. We isolated three putative soybean PR genes from mRNA microarray analysis data of soybean plants inoculated with soybean cyst nematode (SCN: *Heterodera glycines*). Quantitative-PCR analysis revealed that these genes are highly expressed by SCN attack with a similar pattern, but differs in intensity. We also analyzed their responses against various abiotic stresses such as cold, drought, and salt stress. Result of Q-PCR analysis showed that they have different abiotic stress response patterns among them. One of our PR genes is highly induced by salt but not by cold, and drought. Interestingly, all of these genes are expressed in roots only, meaning that they may be involved in defense response against root-related biotic and abiotic stresses such as root attack insects like SCN and salt stress from soil. To identify the function of three putative PR genes, we generated each of the PR genes-suppressed transgenic soybean roots via *Agrobacterium rhizogenes*-mediated transformation. RT-PCR analysis showed that the transgenic roots showed significant reduction of the target genes. Here, we report the results of progressing bioassay experiment for biotic stress resistance, especially for SCN, and abiotic stresses resistance of the putative PR gene-suppressed transgenic soybean roots.

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18 PROTEIN-PROTEIN INTERACTIONS OF THE INOSITOL POLYPHOSPHATE 5-PHOSPHATASES

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Inositol signaling pathways are important for many different developmental and physiological processes in eukaryotes. In plants, inositol signaling is used during drought stress, with the drought hormone, ABA, causing an increase in second messenger Ins(1,4,5)P3 levels. In order to better understand inositol signaling pathways in plants, we have investigated *Arabidopsis* inositol polyphosphate 5-phosphatases (EC 3.1.3.56; At5PTase). There are four so-called Group B 5PTases which each contain multiple WD40 repeats implicated in protein-protein interactions. The WD40 region of 5PTase14 is the closest relative to 5PTase13, sharing 75.3% identity. 5PTase12 and Fra3 have 62.4% and 46.5% identity to 5PTase13, respectively.

Recently, we have shown that 5PTase13 interacts with the energy stress and developmental modulator, SnRK1.1 in vitro. SnRK1.1 has been shown to coordinate the derepression of catabolic metabolism and the suppression of anabolic metabolism, and is involved in developmental regulation as well. We have also shown that 5PTase13 stabilizes SnRK1.1 from proteasomal destruction when plants are grown under low nutrient conditions. We propose to investigate

5PTase WD40 protein complexes by using a tandem affinity purification (TAP) approach. In this approach we will be using the TAPa vector to produce recombinant proteins featuring a myc epitope tag, a histidine tag, and two copies of the IgG binding domain to facilitate optimal purification.

We expect that this purification strategy will validate the 5PTase13 interaction with SnRK1.1, as well as uncover novel protein interactions.

19 A PCR-BASED ASSAY FOR DETECTION OF *PUCCINIA HORIANA* ON CHRYSANTHEMUMS

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Puccinia horiana (Henn.), the causal agent of chrysanthemum white rust, is a pathogen of quarantine status in most countries where *Chrysanthemum x morifolium* cultivars are grown. Current identification protocols for white rust rely upon macroscopic symptom development and microscopic examination of infected leaves for teliospores. Symptoms become visible 7 – 10 days after initial infection under favorable conditions followed by the production of pustules (telia) on the abaxial surface of infected leaves. Infected plants can therefore evade detection before symptoms and fruiting bodies become evident. The aim of this work was to develop a DNA-based assay to for the detection of *P. horiana*. The ribosomal internal transcribed spacer (ITS) region was cloned and sequenced from 14 isolates of *P. horiana* and used to develop primers for both conventional and real-time PCR assays. The *P. horiana*-specific primers could detect as little as 1 ng of DNA in conventional PCR assays and 1 pg in real-time PCR assays. Both assays were capable of detecting *P. horiana* in asymptomatic tissue. The primers were also tested for specificity using DNA isolated from leaf tissue infected with chrysanthemum brown rust, caused *P. tanaceti*, and did not show any cross reactivity.

20 *GUNNERA*: A MODEL SYSTEM TO STUDY PLANT-*NOSTOC* SYMBIOSES

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To address the problem of nitrogen limitation, a remarkably diverse set of land plants have evolved different strategies to enter into symbioses with N_2-fixing cyanobacteria. *Gunnera* is the only group within the flowering plants known to form symbiosis with cyanobacteria. Understanding how *Gunnera* plants manage to attract and maintain cyanobacteria may help us to extend the benefits of biological N_2-fixation to other plants.

Gunnera plants have evolved a unique muciliage-secreting gland on the stem below each leaf which serves to attract and house cyanobacteria. While trying to establish *Gunnera-Nostoc* association in the lab, we discovered that gland development is stimulated by nitrogen deprivation[1]. Upon further investigation, we found that, like other plants, *Gunnera* accumulates flavonoids and carbohydrate when combined nitrogen becomes limited. However, *Gunnera* is able to use these common physiological changes to stimulate gland development. Although cells in the

stem adjacent to the mature glands accumulate starch, cells in the glands are mostly free of starch but rich in soluble sugars.

Cyanobacteria, primarily *Nostoc,* enter *Gunnera* glands in the form of short motile filaments termed hormogonia. Inside the gland, hormogonia manage to enter *Gunnera* cells and revert back to N_2-fixing vegetative filaments. Further hormogonia formation is suppressed in the host environment. To understand how *Gunnera* regulates cyanobacteria development, we tested the effects of flavonoids and sugars on *Nostoc* hormogonia formation. Indeed, the flavonoids naringenin (at 0.1mM) and quercetin (at 0.5mM) as well as metabolizable sugars (sucrose, glucose, and fructose) can inhibit hormogonia formation. Since both sugars and flavonoids are in high concentrations within the *Gunnera* gland, it is likely that these metabolites play important roles in regulating the development of cyanobionts. Currently, we are testing the role flavonoids play in interactions between *Gunnera* and *Nostoc* by knocking down chalcone synthases using RNAi.

[1]Chiu *et al.,* (2005). Nitrogen deprivation stimulates symbiotic gland development in *Gunnera manicata.* Plant Physiology 139:224-230.

21 THE EFFECT OF SOYBEAN HAIRY ROOT MORPHOLOGY ON THE INFECTIVITY OF SOYBEAN CYST NEMATODE

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Soybean cyst nematode (SCN), *Heterodera glycines*, is a serious pest of soybean, causing root damage and lower productivity amounting to one billion dollars in losses each year in the United States. One possible approach to broadening resistance of soybean to SCN is to silence genes critical to SCN survival through the expression of RNAi in the soybean root. Upon feeding, SCN would ingest the RNAi and the gene would be silenced. Another approach is to introduce full-length soybean cDNAs encoding resistance genes into soybean, then screen for resistance. However, this is very labor intensive by the current methods. We developed a non-tissue culture method to rapidly screen vector constructs using composite plants. The soybean shoot is excised and the base is transformed using *Agrobacterium rhizogenes* carrying the gene-of-interest in a binary vector that contains GFP as a selectable marker. Transformation of soybean roots using *A. rhizogenes* produces a 'hairy root' phenotype. The 'hairy root' morphology differs quite a bit from wild type roots. Hairy roots are much narrower, and are vastly more branched. Also there are fewer root hairs on the hairy roots. The cross-section of the root, though, shows similar cellular structure organization in both hairy root and wild type phenotypes.

In this study we compare *A. rhizogenes* transformed roots with wild type soybean roots to determine if the 'hairy root' phenotype has an effect on nematode infection. Transformed and untransformed soybean roots were infected with soybean cyst nematode juveniles and were monitored for the infectivity over a time course of 3 days, 8 days, and 30 days. Number and size of juveniles were monitored. Also root width and cell structure was compared with control plants.

Three days after infection with SCN, one wild type control and one plant transformed with A. rhizogenes containing our pRAP17 vector that expresses green fluorescent protein were stained with acid fuchsin to observe the juveniles inside the root. The size of the juveniles was comparable between normal and transgenic plants. The number of juveniles inside the wild type root was more than two times higher than in the transgenic roots. For both sets of roots, the juveniles preferred entering within 2 cm of the root tip, but the normal plants had many juveniles entering within 5 cm

of the root tip. By eight days, the number of maturing juveniles in the roots was equivalent. The width of the juveniles growing in transgenic hairy roots was 41 percent narrower on average than juveniles in wild type roots. At thirty days, the remaining 18 plants for each were scored for the number of emerged, mature females. The mature female count on the transgenic, hairy roots was only 11 percent lower than the wild type control. In conclusion, there is a significant effect of hairy root morphology to the infectivity and growth of SCN. Hairy roots with candidate nematode-resistance constructs should be compared to vector-only control hairy roots to monitor expression of resistance.

22 POST TRANSCRIPTIONAL GENE SILENCING OF A PLANT PARASITIC-ROOT KNOT-NEMATODE IN SOYBEAN

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Root-knot nematodes (*Meloidogyne incognita*; RKN) are sedentary endoparasites with a broad host range, which includes economically important crop species. This nematode can damage and suppress yields of susceptible soybean cultivars by as much as 90% and yields of resistant cultivars by 40 %.

RNA interference (RNAi) has recently been demonstrated to silence genes in plant parasitic nematodes. It is a potentially powerful investigative tool for the genome-wide identification of gene function that should help improve our understanding of plant parasitic nematodes. Therefore, the present investigation was conducted with two main objectives. The first objective was to study the gene expression and the product functions of those genes that are turned on during infection in comparison to the noninfected controls. The second objective was to design RNAi constructs to silence specific nematodes genes that are expected to have essential functions in the RKN life.

In this work, RKN genes having high similarity with essential cyst nematode and *C. elegance* genes were identified using BLAST. Seven RKN genes were chosen according to their expected essential function in the life cycle. We amplified the seven genes using gene specific primers and RKN cDNA as template. The amplified DNA was cloned into the vector pENTR, and then sequenced for confirmation. Finally, the insert was moved into a pRAP vector and transformed into *Agrobacterium rhizogenes.* The presence of the insert and plasmid in *A. rhizogenes* was confirmed using PCR. In the future, we will transform soybean to obtain roots expressing RNAi to silence these RKN genes. The transformed roots will be recognized by the presence of green fluorescent protein. The transformed roots will be challenged with RKN to determine if the construct interferes with the life cycle of RKN and if it can be used to broaden resistance of soybean against RKN.

23 DETECTION OF MISIDENTIFIED PLANTS IN INTERNATIONAL COCOA GENEBANK, TRINIDAD

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Theobroma cacao L. is an important crop plant grown commercially in several tropical countries throughout the world. This crop originated in the Amazonian basin of South America and diverse germplasm is maintained in various living collections of trees due to the poor viability of seeds in storage. Each clonal accession is usually represented by multiple trees, however, management of these germplasm collections is commonly complicated by misidentification, which may be due to scion die-back in grafted plants or introduction of mislabeled individual trees into a clonal germplasm group. Towson University and the University of West Indies are investigating the prevalence of misidentified trees in the International Cocoa Genebank, Trinidad (ICG,T) that has over 2,300 accessions, each represented by up to 16 trees per plot. Using the 15 simple sequence repeat (SSR) DNA genotyping probes developed as international standards for *Theobroma cacao* L. (Zhang et al., 2006) microsatellite profiles for individual trees from the ICS accession group were analyzed. Initially twelve genotypes were tested and only one misidentified tree was detected among these. Based upon the low incidence of misidentified plants we cannot reliably extrapolate to the entire germplasm collection, but it appears the incidence of misidentification is low. Further studies on a larger group of accessions/genotypes will provide additional insight on the extent of mislabeled clones in this collection.

24 GLUCOSINOLATES AND THE INFECTION OF *ARABIDOPSIS THALIANA* WITH *TURNIP MOSAIC VIRUS* (TuMV)

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Glucosinolates are secondary metabolites present in plants of the Brassicales order that are known to function in the chemical defense against general herbivory. The role of glucosinolates and their activating enzyme myrosinase in pathogen defense has not been well-characterized but is presumed to be associated with innate immunity. We are developing a pathosystem involving *Arabidopsis thaliana* and the potyvirus *Turnip mosaic virus* (TuMV, + ssRNA) to clarify the role of glucosinolates in the plant response to viral infection.

TuMV was selected as a pathogen based on a screening of transcriptome datasets of *A. thaliana* generated after infections by various pathogens. The 50+ genes involved in the glucosinolates-myrosinase system are well-documented in *A. thaliana* and comprise at least four clusters associated with the biosynthetic and signaling pathways for glucosinolate formation and activation. Susceptible plants infected with TuMV revealed the down-regulation of 9 out of 20+ members of the glucosinolate biosynthesis gene cluster (Yang et al, 2007) indicating a possible role for glucosinolates in the infection process.

To assess the role of the glucosinolates-myrosinase system in the TuMV infection process, we are using a single local isolate of TuMV to infect various four-week-old accessions and mutants of *A. thaliana.* By 14 days following mechanical inoculation with TuMV, the plant lines displayed various levels of reduced stature, leaf necrosis, reduced apical dominance, absence of bolts, curled bolts, aborted flowers, developmentally abnormal reproductive organs, and mosaic pattern on leaves. Disease symptoms were generally more severe on transgenic lines containing reduced levels of glucosinolates including the appearance of veinal necrosis on one plant line. In all cases the presence of the virus was detected in infected plants using tissue immunoassays. We are developing inoculation protocols to assess plant susceptibility quantitatively, as well as phenotypically in plant lines with different glucosinolate levels. To monitor viral titer and the spatial and temporal patterns of infection, we will employ a PCR-based quantitative assay and immunoassays using anti-TuMV polyclonal antibodies.

25 BIOSYNTHESIS AND REGULATION OF STEROIDAL GLYCOALKALOIDS IN *SOLANUM CHACOENSE*

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Steroidal glycoalkaloids (SGAs) are secondary metabolites produced by approximately 350 species of the Solanaceae family including potato and tomato. They are organoleptic at low concentrations but at higher concentrations are toxic to insect pests and mammals. The SGAs of potato comprise one of two major classes of secondary metabolites formed by the mevalonate branch of the isoprenoid biosynthetic pathway. Squalene synthase (SQS) is a key branch point enzyme in SGA biosynthesis. To identify the role of SQS in the regulation of SGA accumulation, we are using the wild potato species *Solanum chacoense* to elucidate the distribution of SGAs and to characterize the gene coding for squalene synthase isolated from an accession that has higher levels of SGAs than cultivated potato.

Tissues were harvested at specified developmental stages and analyzed by HPLC for their profile and content of SGAs. A cDNA coding for squalene synthase was generated using RNA extracted from leaves of *S. chacoense*, sequenced and used to map steady-state transcript levels in the various tissues of the developmental profile. A tissue profile of the mature plant reveals a broad range of SGA concentrations (6 to 67 µmol/g Dry Weight) from the lowest levels in the roots and stems, higher levels in generative tissues such as auxiliary shoots, flowers and above-ground stolons (36-55 µmole/g DW), to the highest in the shoot meristem. With plant maturation, newly emergent leaves and associated stems have higher SGA levels than their older counterparts. The development of below-ground stolons shows a transition in SGA profile and concentration that spans the simple profile (two SGAs) and low concentration of the roots to the more complex profile (>10 SGAs) and higher levels of the shoot meristem.

We used oligonucleotide primers based on the nucleotide sequence of potato SQS to PCR-amplify a near full-length cDNA derived from leaf RNA of *S. chacoense.* The deduced amino acid sequence shows >75% identity with SQS from the closely-related *S. tuberosum* and about 63% identity with other angiosperm SQSs. SQS primers have been used to quantify steady-state mRNA levels and compare them with SGA levels in *S. chacoense* tissues.

In future work, we will clone and characterize the full squalene synthase gene and isolate a clone from an accession that has reduced SGA accumulation. These tools will be used to characterize SQS expression and enzymatic activity, and to identify nucleotide polymorphisms for association mapping of SGA content in potato.

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

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The acyclic C_{11}-homoterpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) is a major constituent of volatile blends emitted from herbivore-damaged foliage of many plant species. We have found that DMNT is also emitted from roots of *Arabidopsis thaliana* after treatment with the defense hormone jasmonic acid (JA) suggesting defense functions of DMNT in plant roots. It has been demonstrated that DMNT is derived from the sesquiterpene alcohol (*E*)-nerolidol. The enzymatic steps involved in the conversion of (*E*)-nerolidol to DMNT are not understood. However, participation of CytP450 enzymes and peroxidase-type enzymes has been suggested based on mechanistically similar C-C-bond cleavage reactions in several other secondary metabolite biosynthetic pathways. We are using a combined biochemical and functional genomics approach in order to understand the biosynthetic pathway leading to DMNT formation. We have studied the involvement of CytP450 enzymes in oxidative degradation of (*E*)-nerolidol by application of enzyme specific inhibitors in *Arabidopsis* root cultures together with JA treatment. Application of different inhibitors of Cytp450s such as clotrimazole and miconazole led to highly decreased DMNT formation which strongly suggests a participation of CytP450 enzymes in nerolidol degradation. We are also establishing microarray datasets of JA-treated *Arabidopsis* roots in order to pinpoint candidate genes involved in oxidative degradation of nerolidol. Furthermore, we are investigating the subcellular organization of root-specific DMNT formation.

27 CONSTITUTIVE AND INDUCED VOLATILE TERPENE FORMATION IN *ARABIDOPSIS* ROOTS IN INTERACTION WITH THE ROOT HERBIVORE *BRADYSIA* SPP. (FUNGUS GNAT)

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Plants release a bouquet of volatile organic chemicals into the environment that can mediate ecological interactions. Volatile terpenes represent a major class of plant volatiles that play important roles in direct plant defense against herbivores and pathogens but they also serve as indirect defense compounds by attracting natural predators of plant herbivores. Though research in the past 10 to 15 years has deepened our understanding of the biology of volatile terpenes in aboveground plant tissues, our knowledge of the metabolism of volatile terpenes emitted from roots and their role in root-organism interactions is still limited. Using *Arabidopsis thaliana* as a model plant, we investigate the formation and function of volatile terpenes in plant roots. We have detected the constitutive emission of monoterpene- (C_{10}), sesquiterpene- (C_{15}) and diterpene- (C_{20}) volatiles from *Arabidopsis* roots grown under axenic and hydroponic culture conditions. Using a reversed genetics approach, we have identified a terpene synthase that is responsible for root-specific diterpene formation, and we are currently analyzing its cell-specific and subcellular regulation and possible function in root defense. In order to further investigate the possible functions of these volatiles belowground we have established a system to study *Arabidopsis* root

herbivory, using an aeroponic root culture system and the common root herbivore generalist *Bradysia* spp. (fungus gnat) as an experimental model. We are in the process of analyzing changes in root terpene formation in response to *Bradysia* larval feeding. To determine the effect of individual volatiles on *Bradysia* feeding, bioassays using transgenic A*rabidopsis* plants with modified terpene volatile profiles will be conducted.

28 VOLATILE TERPENE FORMATION IN *ARABIDOPSIS* ROOTS IN INTERACTION WITH THE SOIL-BORNE PATHOGEN *PYTHIUM IRREGULARE*

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Volatile terpenes have been observed as defense molecules in plant-environment interactions, which have been extensively studied in aerial parts of plants. However, little knowledge has been gained on the synthesis and function of volatile terpenes in plant roots. Using *Arabidopsis thaliana* as a model, we have found constitutive emission of mono-(C10), sesqui-(C15) and di-terpenes (C20) in roots in various culture systems. Treatment of *Arabidopsis* roots with the oomycete root pathogen *Pythium irregulare* elicits the release of the C11-homoterpene DMNT (4,8-dimethylnona-1,3,7-triene) with highest emissions during the early stage of infection at which germ tubes penetrate the root epidermis. Since homoterpenes are involved in induced defense responses in leaves, we speculate that DMNT might also play a role in the defense against soil-borne pathogens. This hypothesis is supported by preliminary results showing that DMNT retards *P. irregulare* growth *in vitro.*

We are currently in the process of characterizing the enzymatic steps for DMNT formation in *Arabidopsis* roots by identifying terpene synthase (*TPS*) gene(s) responsible for the first committed step in the synthesis of DMNT. Furthermore, to understand the regulation of DMNT emission, we investigate the role of defense signaling pathways in response to *Pythium* infection. Preliminary results indicate that DMNT formation is dependent on the biosynthesis or perception of the defense-related hormones jasmonate and ethylene. In addition, we have observed that *Pythium* infection elicits the formation of reactive oxygen species such as superoxide and peroxide at root tips prior to the induced emission of DMNT. Further studies on the synthesis and function of DMNT will aid in our understanding of root chemical defense against soil-borne pathogens.

29 EXPRESSION OF THE CASSAVA VEIN MOSAIC VIRUS (CSVMV) PROMOTER IN TRANSGENIC SOYBEAN (GLYCINE MAX) PLANTS

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Plant pararetrovirus promoters have been isolated and used in previous studies to express foreign genes in transgenic plants. The cauliflower mosaic virus 35S (CaMV) promoter is one of the pararetrovirus promoters that has been widely used in various plant biotechnology experiments. However, according to previous studies, when the 35S CaMV promoter was fused with the green fluorescent protein (GFP) reporter gene and analyzed in *Arabidopsis thaliana* roots infected with *Heterodera schachtii*, there was significant decline of GFP expression suggesting that the 35S CaMV promoter was down regulated in the presence of *Heterodera schachtii.* To overcome the

limitations of CaMV, we cloned and are testing the cassava vein mosaic virus (CsVMV) promoter. The CsVMV is a double stranded DNA pararetrovirus that infects cassava in Brazil. Previous studies have demonstrated the capability of the CsVMV promoter in driving significantly high levels of heterologous gene expression in different plants and in protoplasts. We tested the CsVMV promoter to determine its pattern and level of expression in soybean (*Glycine max*) transgenic roots. The CsVMV promoter was chemically synthesized by Integrated DNA Technologies, Inc., and cloned it into the Gateway® plasmid pZErO-2 vector and amplified using polymerase chain reaction (PCR). The CsVMV promoter was moved into the pENTR vector and the presence of the promoter was verified by PCR and Big-dye terminator cycle sequencing. To test the expression of the CsVMV promoter, we inserted the promoter into PRAP40 expression vector that contains the enhanced GFP (EGFP) as a reporter gene. The final recombinant construct was transformed by electroporation into *Agrobacterium rhizogenes* strain K599 and infected into soybean stems. After four to six weeks, non-transformed roots were trimmed and roots expressing EGFP (enhanced green fluorescent protein) were retained. We observed significant EGFP fluorescence activity in roots indicating that the CsVMV promoter expresses well in soybean roots. In the future we will challenge the roots with *Heterodera glycines* to determine if the CsVMV promoter expresses well after nematode infection.

30 A FUNCTIONAL ANALYSIS OF THE PYRIMIDINE CATABOLIC PATHWAY IN *ARABIDOPSIS*

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In plants, catabolism of pyrimidine nucleotides occurs via a three-step reductive pathway in which uracil (Ura) is degraded to ß-alanine, CO_2 and NH_3 through sequential activities of dihydropyrimidine dehydrogenase (EC 1.3.1.2, PYD1), dihydropyrimidinase (EC 3.5.2.2, PYD2) and ß-ureidopropionase (EC 3.5.1.6, PYD3). A proposed function of this pathway, in addition to maintenance of pyrimidine homeostasis, is the recycling of pyrimidine N to general N metabolism. *PYD* expression and catabolism of [2-^{14}C]-Ura are markedly elevated in response to N limitation in wild type plants, which can utilize uracil as a sole N source. We used *PYD1, PYD2* and *PYD3* knockout mutants to perform a functional analysis of this pathway in Arabidopsis (*Arabidopsis thaliana*). The *pyd* mutants exhibited no obvious phenotype under optimal growing conditions. *pyd2* and *pyd3* mutants were unable to catabolize [2-^{14}C]-Ura or to grow on Ura as a sole N source. In contrast, catabolism of Ura was reduced by only 40% in *pyd1* mutants, compared with wild type plants, and *pyd1* plants grew nearly as well as wild type plants with a Ura N source. These results confirm both PYD1 function and the possible existence of an alternative mechanism for Ura degradation in this plant. Localization of PYD-GFP fusion proteins in the plastid (PYD1), secretory system (PYD2) and cytosol (PYD3) suggest potentially complex metabolic regulation of the catabolic pathway.

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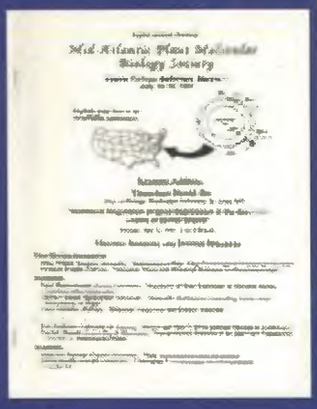

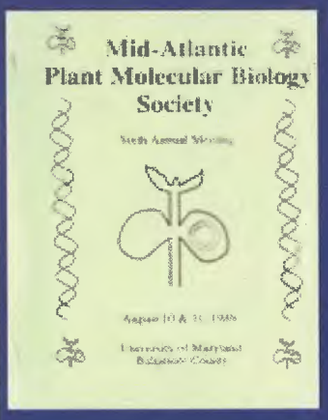

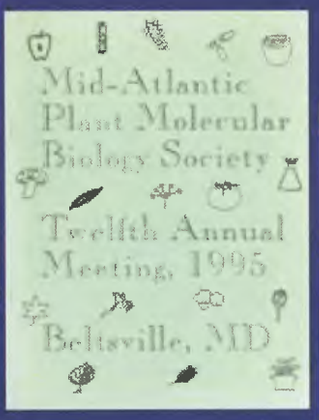

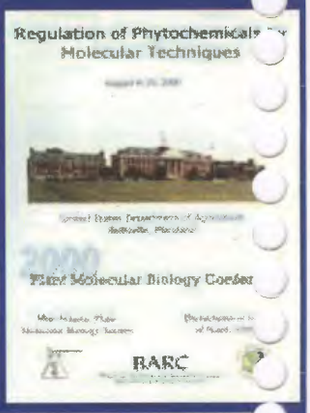

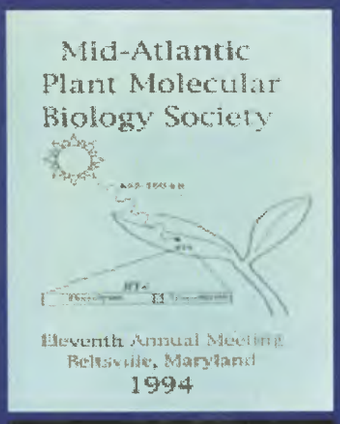

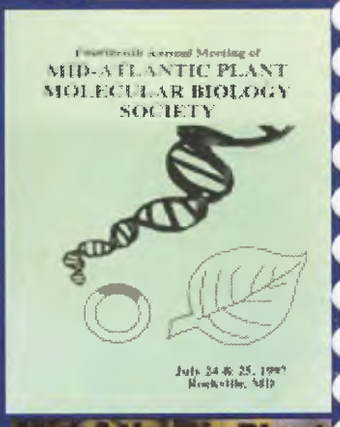

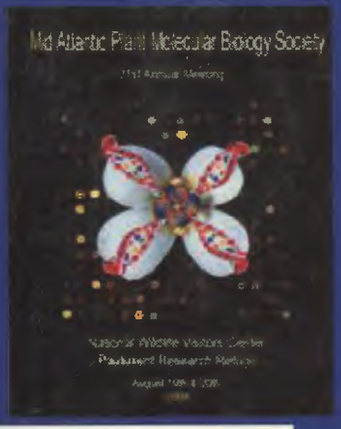

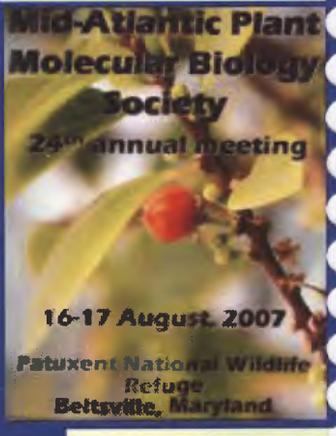

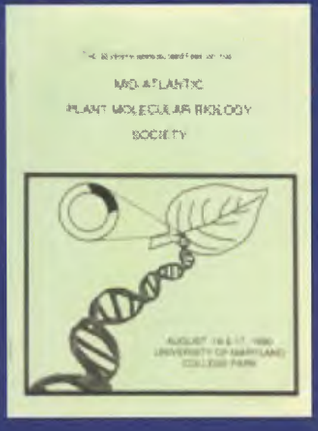

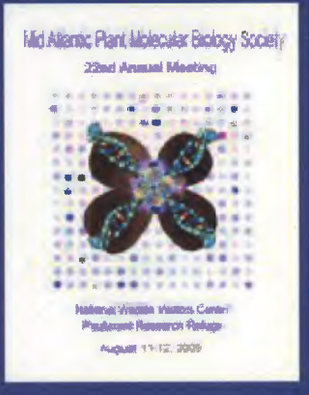

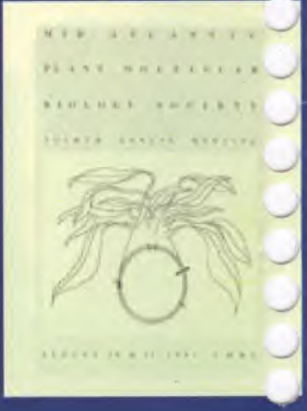

25TH ANNIVERSARY

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