ACKNOWLEDGEMENTS

THANK YOU VERY MUCH…

For generous financial support, the American Society of Plant Biologists.

For support of the Keynote Symposium and generous support-in-kind, Dean John Kirby and the University of Rhode Island College of the Environment and Life Sciences.

For donation of books for the raffle, Susan McGlew and Sinauer Associates, Inc., Publishers.

For assistance with conference preparation, Denise Brown, Robin White, Cindy Messier and Meredith Silvia.

For donations to the student travel fund:

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SCHEDULE

SATURDAY, MARCH 29		
Time	**Activity**	**Location**
11:00-12:45	Registration and poster set up	CBLS Atrium
12:45-1:00	Opening: Alison Roberts Welcome: Dean John Kirby	CBLS 100
1:00-1:50	**S1** Rowan Sage, University of Toronto The evolution of C4 photosynthesis	CBLS 100
1:50-2:40	**S2** Jill Preston, University of Vermont Evolution of flowering time in seasonally cold-adapted grasses	CBLS 100
2:40-3:10	**COFFEE BREAK**	CBLS Atrium
3:10-4:00	**S3** Chris Lane, University of Rhode Island Red algal parasites: The genomic consequences of going rogue	CBLS 100
4:00-4:15	Crispin Taylor, American Society of Plant Biologists News from the Society	CBLS 100
4:15-6:15	**POSTER SESSION AND RECEPTION** Even numbered posters: 4:15-5:15 Odd numbered posters: 5:15-6:15	CBLS Atrium
6:15-6:30	**BOOK RAFFLE** Susan McGlew, Sinauer Associates	CBLS Atrium
6:30-6:45	Adjourn to University Club	
6:45-9:15	**BANQUET AND CASH BAR**	University Club
9:15-10:00	Executive Committee Meeting	CBLS 152

SUNDAY, MARCH 30		
7:45-8:30	**COFFEE AND PASTRIES**	CBLS Atrium
8:30-8:45	**T1** Madelaine Bartlett, University of Massachusetts Amherst Changing B-class MADS box protein-protein interactions across evolutionary time: when, how, and why?	CBLS 100
8:45-9:00	**T2** Jeffrey Bibeau[G], Worcester Polytechnic Institute Computational and experimental FRAP analyses of myosin XI dependent vesicular transport shows coupling with F-actin in polarized cell growth	CBLS 100
9:00-9:15	**T3** Maegan Gagne[G], University of New Hampshire Nitrogen assimilation in shrub willow fertilized with different forms of nitrogen via foliar and soil application	CBLS 100
9:15-9:30	**T4** Joshua Gendron, Yale University Comprehensive analysis of circadian clock regulated protein degradation using a decoy F-box strategy	CBLS 100
9:30-9:45	**T5** Francine Carland, Yale University Small-molecule dissection of vein patterning in *Arabidopsis*	CBLS 100
9:45-10:15	**NEASPB Business Meeting**	CBLS 100
10:15-10:30	**COFFEE BREAK**	CBLS Atrium
10:30-10:45	**T6** Peter Melcher, Ithaca College A new approach to measure hydraulic resistance in plants	CBLS 100
10:45-11:00	**T7** Linda Kirchner[G], Northeastern University Identification and characterization of diacylglycerol acyltransferase type-1 (DGAT1) from *Chlorella vulgaris*	CBLS 100
11:00-11:15	**T8** Matthew Mattozzi, Harvard Medical School The chloroplast as a platform for metabolic engineering	CBLS 100
11:15-11:30	**T9** Wil Prall[U], The College of New Jersey Phylogenetic and functional analysis of the CYP72A enzymes contributing to secondary metabolism in flowering plants	CBLS 100
11:30-11:45	**T10** Noreen Rizvi[G], Northeastern University An optimized transformation method for estrogen-inducible GFP expression in *Catharanthus roseus* hairy roots	CBLS 100
11:45-12:00	**T11** Pamela Weathers, Worcester Polytechnic Institute Developing a whole plant *Artemisia annua* antimalarial therapeutic	CBLS 100
12:00-12:15	Sandra Smieszak, ASPB Ambassador ASPB Ambassador Program	CBLS 100
12:15-12:30	**Award Presentation**	CBLS 100
12:30	Adjourn	

G=Graduate student speaker U=Undergraduate student speaker

POSTER SESSION

BIOCHEMISTRY		
P1	Abdullah, H.[G] and Dhankher, O.P.	Transcriptome profiling of genes involved in triacylglycerol biosynthesis in developing seeds of *Camelina sativa*
P2	Hoskin, J.[U] and Hrabak, E.	Identification of palmitoyltransferase mutants in *Arabidopsis thaliana*
P3	Barchi, B. [G], Turlapati, S.A., Shao, L., Majumdar, R. and Minocha, S.	Effect of genetic manipulation on polyamines biosynthetic pathway (glutamate-ornithine-arginine/proline) in *Arabidopsis thaliana*
P4	Melloni J. [G] and Minocha S.	Manipulating polyamine biosynthesis to increase carbon sequestration in *Dunaliella* spp.
GROWTH AND DEVELOPMENT		
P5	Famiglietti, K.M.[U], Duncan, L.H., Prabhu, R. and Silady, R.A.	Identification of enhancers and suppressors of *gravitropism defective* (*grv*)
P6	Almeida R.A.[G] and Chandlee J.M.	Candidate gene discovery for primary regulatory genes of soybean senescence using a map based approach
P7	Carini, A.B.[U], Gabow, K. [U], Bergenfeld, D.[U] and Owen, T.P.	Developmental and morphological changes in *Nepenthes* peristomal nectary glands
P8	Chamberlin, C.A.[G] and Gendron, J.M.	Analysis of daily ubiquitylome dynamics to determine circadian control of global protein degradation
P9	Feke, A.M.[G] and Gendron, J.M.	Targets of clock proteins FKF and LKP: A first glance
P10	Chou, H.[G], Zhao, Y. and Berkowitz, G.A.	Linking Ca^{2+} to the brassinosteroid signal transduction cascade: molecular steps that modulate Br responsive gene expression
P11	Flaman, L.[U], Milkey, K., Thompson, M. and Hrabak, E.	Identifying mutation sites in protein phosphatase a causing sodium-dependent root skewing in *Arabidopsis thaliana*
P12	Ma, Y., Zhao, Y.C., Thatiparth, A. and Berkowitz, G.A.	Examination of the three putative functional residues in the putative guanylyl cyclase domain in PEPR and BRI
BIOTECHNOLOGY		
P13	Fei, L.[G] and Weathers, P.J.	One-step micropropagation from leafy tissue into rooted plantlets in a mist bioreactor
P14	Hague, J., Tilelli, M., Cunha, D., Nelson, K., Kausch, A., Heffelfinger, C., Moreno, M. and Dellaporta, S.	*In situ* embryo rescue as a novel method for recovery of non-GMP hybrids from wide crosses
P15	Etemadi, F.[G], Masoud H., Hamid M. and Baoshan X.	Distribution of L-DOPA in different parts of fava beans
P16	Van Beaver, L.[U]	Production of decaffeinated tea through genetic engineering

P17	Wang, S.[G], Towler, M. and Weathers, P.	Effect of roots on artemisinin and flavonoid production in shoots of *Artemisia annua*
P18	Shaw, S.E.[G] and Lee-Parsons, C.W.T	Investigating the limiting step in the transcription of the vindoline pathway in *Catharanthus roseus* using RT-PCR
P19	Weaver, J.[G], Cram, E. and Lee-Parsons, C.	Promoter analysis of *ZCT*, a key repressor of alkaloid production in *C. roseus*
P20	Phillips, A.[HS]	Accelerating bioenergy: the effect of NPK fertilizer regimes on the recovery of transplanted clones of switchgrass (*Panicum virgatum* cv. alamo) atlantic coastal panic grass (*Panicum amarum) and* genetically modified switchgrass (*Panicum virgatum* cv. Alamo)
P21	Wirshing, A.[G], Kirchner, L., Cram, E. and Lee-Parsons, C.	Development of genetic engineering techniques in microalgae for biofuel production
P22	Tilelli, M.[G], Nelson, K., Deresienski, A., Hague, J. Cunha, D. and Kausch, A.	Recovery of intraspecific and interspecific hybrids in switchgrass (*Panicum virgatum* L.) via a transgenic herbicide resistance selectable marker
P23	Deng, Y.[G], Gagnon, M., Minocha, S. and Vasudevan, P.	Transgenic expression of *Thermomyces lanuginosus* and *Candida antarctica* lipases in plants for the enzymatic production of biodiesel
CELL BIOLOGY		
P24	Furt, F., Callahan, K.P., Bibeau, J.P. and Vidali, L.	High resolution imaging of myosin XI-driven vesicles on actin filaments in the moss *Physcomitrella patens*
P25	Griffin-Nolan, R.J.[G], Sattarzadeh, A., Hanson, M.R. and Owens, T.G.	Investigating the role chloroplast movements play in non-photochemical quenching in *Arabidopsis thaliana*
P26	Scavuzzo-Duggan, T.[G], Slabaugh, E., Sethaphong, L., Yingling, Y., Haigler, C.H. and Roberts, A.W.	Functional analysis of CESA structural features using a genetic complementation assay in *Physcomitrella patens*
P27	Berry, E.[U], Scavuzzo-Duggan, T., Tran, M.L. and Roberts, A.W.	Localization of cell wall polysaccharides in *Physcomitrella patens*
P28	Tran, M.L.[G] and Roberts, A.W.	Cellulose up-regulation under osmotic stress in *Physcomitrella patens*
EDUCATION		
P29	Donovan, S., Burgess, Z. and Roberts, A.	Building skills for inquiry in biology: using micropipetters and standard curves
P30	Kausch, A., Hague, J., Tilelli, M., Cunha, D., Mellen, L., Kingsborough, B., Johnson, A., Perretta, L. and Nelson, K.	Agricultural biotechnology and GMOs: Informing the debate—a one credit educational online module
P31	Nelson, K., Hague, J., Tilelli, M., Cunha, D., Mellen, L., Kingsborough, B., Johnson, A., Perretta, L. and Kausch, A.	Laboratory internships in plant biotechnology: An inquiry-driven experiential learning opportunity in agricultural biotechnology

ENVIRONMENTAL PHYSIOLOGY		
P32	Ablordeppey, K.K.[G], Khadka, A. and Dhankher, O.P.	A novel γ-glutamyl cycle in plants and its role in providing tolerance to oxidative stress via glutathione homeostasis
P33	Hudzik, C.[U], Baldo, A., Wilson, M., Shortt, K., Taylor, C.G., Haswell, E. and Marella, H.H.	Do mechanosensitive channels function in root-knot nematode parasitism?
P34	Ma, C.[G], Chhikara, S., Xing, B., Musante, C., White, J.C. and Dhankher, O.P.	Gene regulations and antioxidant enzyme responses in defense system of *Arabidopsis thaliana* to nanoparticle cerium and indium oxide exposure
P35	Mercadante, C.[U], DuLong, C., Fester, T., Taylor, C.G. and Marella, H.H.	Exploring nematode-induced changes in transporter gene expression
P36	Turlapati, S.A., Minocha, R., Eren, A.M., Ramsdell, J.S. and Minocha, S.	Previously undiscovered effects of N-fertilization on diversity of soil acidobacteria at Harvard Forest, MA
P37	Sarver, K.S.[G] and Minocha, S.C.	Transgenic upregulation of polyamine biosynthesis in willow and hybrid *Populus* to enhance nitrogen and carbon assimilation
P38	Petersen, N.[U], Ryan, M., Flaman, L., Milkey, K., Thompson, M. and Hrabak, E.	A screen to identify sodium-induced root skewing mutants in *Arabidopsis thaliana*
P39	Soler, A.[U], Gidugu, S., Hendy, O., Piatt, T., Prall, W., Yerram, N. and Thornton, L.E.	Molecular genetic and biochemical analysis of the stress response activity of CYP72A enzymes from *Arabidopsis*

G=Graduate student U=Undergraduate student HS=High school student

DIRECTORY OF PARTICIPANTS AND ABSTRACT NUMBERS

S=Symposium, P=Poster, T=Contributed talk

First Name	Last Name	Abstract numbers	First Name	Last Name	Abstract numbers
Hesham	Abdullah	P1	Carolyn	Lee-Parsons	P18,P19,P21
Kenny	Ablordeppey	P32	Yi	Ma	P12
Robert	Almeida	P6	Chuanxin	Ma	P34
Boubker	Barchi	P3	Heather	Marella	P33,P35
Madelaine	Bartlett	T1	Matthew	Mattozzi	T8
Dana	Bergenfeld	P7	Peter	Melcher	T6
Elizabeth	Berry	P27	Joseph	Melloni	P4
Jeffrey	Bibeau	T2,P24	Courtney	Mercadante	P35
Alison	Carini	P7	Subhash	Minocha	P3,P4,P23,P36,P37
Francine	Carland	T5	Rakesh	Minocha	P36
Catherine	Chamberlin	P8	Kimberly	Nelson	P14,P22,P30,P31
Joel	Chandlee	P6	Page	Owen	P7
Hsuan	Chou	P10	Nicole	Petersen	P38
Erin	Cram	P19,P21	Alexander	Phillips	P20
Ye	Deng	P23	Rajkumar	Prabhu	P5
Om Parkash	Dhankher	P1,P32,P34	Wil	Prall	T9,P39
Shannon	Donovan	P29	Jill	Preston	S2
Casey	DuLong	P35	Noreen	Rizvi	T10
Leighton	Duncan	P5	Alison	Roberts	P26,P27,P28,P29
Fatemeh	Etemadi	P15	Rowan	Sage	S1
Kirsten	Famiglietti	P5	Kara	Sarver	P37
Liwen	Fei	P13	Tess	Duggan	26,27
Ann	Feke	P9	Sydney	Shaw	P18
Lisa	Flaman	P11,P38	Rebecca	Silady	P5
Fabienne	Furt	P24,P28,T2	Amanda	Soler	P39
Kyra	Gabow	P7	Megan	Thompson	P11,P38
Maegan	Gagne	T3	Leeann	Thornton	P39,T9
Joshua	Gendron	T4,P8,P9	Michael	Tilelli	P14,P22,P30,P31
Sundeep	Gidugu	P39	Mai	Tran	P27,P28
Robert	Griffin-Nolan	P25	Swathi	Turlapati	P3,P36
Joel	Hague	P14,P22,P30,P31	Laura	Van Beaver	P16
Oliver	Hendy	P39	Luis	Vidali	P24,T2
Judith	Hoskin	P2	Sibo	Wang	P17
Collin	Hudzik	P33	Pam	Weathers	T11,P13,P17
Albert	Kausch	P14,P22,P30,31	Jessica	Weaver	P19
Arogya	Khadka	P32	Alison	Wirshing	P7,P21
Linda	Kirchner	T7,P21	Nikitha	Yerram	P39
Chris	Lane	S3			

ABSTRACTS

SYMPOSIUM: EVOLUTION OF PHYSIOLOGICAL PROCESSES

S1
THE EVOLUTION OF C_4 PHOTOSYNTHESIS
Sage R.F.
Department of Ecology and Evolutionary Biology, University of Toronto

C_4 photosynthesis is one of the most convergent of evolutionary phenomenon in the biological world, with at least 65 independent origins. Evidence from these lineages consistently indicates that the C_4 pathway is the end-product of a series of evolutionary modifications to recover photorespired CO_2 in environments where Rubisco oxygenation is high. Phylogenetically-informed research indicates that repositioning of mitochondria in the bundle sheath is one of the earliest steps in C_4 evolution, as it may establish a single-celled mechanism to scavenge photorespired CO_2 produced in the bundle sheath cells. Elaboration of this mechanism leads to the two-celled photorespiratory concentration mechanism known as C_2 photosynthesis, commonly observed in C_3-C_4 intermediate species. C_2 photosynthesis enhances photosynthetic capacity in very hot environments favoring high rates of photorespiration, and provides a stable intermediate phase between the C_3 and C_4 conditions. In doing so, it establishes the platform on which the C_4 photosynthetic pathway can be assembled via the upregulation of the enzymes and regulatory elements that make-up the C_4 metabolic cycle.

S2
EVOLUTION OF FLOWERING TIME IN SEASONALLY COLD-ADAPTED GRASSES
Preston, J.C.
Department of Plant Biology, University of Vermont

Over the last 50 million years, the Earth has experienced major cooling events, resulting in expansion of the northern and southern temperate zones, and creation of a novel boreal zone. Although most plant species have remained in sub-tropical to tropical regions, a large number of species are now distributed in regions that experience seasonal cold to freezing conditions, suggesting the evolution of traits that allow plants to avoid or tolerate winter. Vernalization is the process by which an extended period of cold readies responsive plant species to respond to inductive flowering time signals, and is a critical adaptation to avoid winter flowering. Data suggest that vernalization responsiveness (VR) evolved multiple times independently within angiosperms through the cooption of related genes. However, the evolutionary lability of VR on a more recent timescale is less well understood. We are investigating the timing and number of evolutionary origins of VR in the cereal-containing grass subfamily Pooideae. Preliminary data suggest that VR maps to the base of Pooideae, concomitant with Cenozoic supercooling. However, the genetic basis of pooid VR suggests more limited conservation, supporting either modification of an ancestral VR pathway or multiple independent trait origins.

Acknowledgements: I would like to thank my Norwegian collaborators Siri Fjellheim, Simen Sandve, and Marian Schubert, and members of my lab Meghan McKeown, Stacy Jorgensen, and Eliszabeth Graves. This work was funded by a USDA-HATCH fund to JCP.

S3

RED ALGAL PARASITES: THE GENOMIC CONSEQUENCES OF GOING ROGUE

Lane, C.E.
Department of Biological Sciences, University of Rhode Island

Parasitism is a life strategy that has independently evolved countless times throughout the eukaryotic tree of life, however most parasitic lineages are distantly related from a free-living taxon making comparative studies difficult. Red algal parasites, however, commonly share a recent common ancestor with their host, earning them the moniker “adelphoparasites” (Adelpho: Greek for “kin”). The close relationship between parasite and host provides an ideal framework to study the genomic consequences of an organism shifting from a free-living to a parasitic life strategy. In typical eukaryotic parasites non-essential genes are lost, as they rely on a host for energy and nutrition. Red algal parasites have never been examined at the genome level and mechanisms for their lifestyle change are unknown. To gain insight into the evolution of parasitism we have conducted a genomic survey of two parasite and host pairs. Genomic data from these pairs and a comparison to the genome of the green algal parasite, *Helicosporidium*, will be presented. The implications for the evolution of parasitism will be discussed, including the loss of genes related to photosynthesis in these parasites.

POSTER SESSION

P1

TRANSCRIPTOME PROFILING OF GENES INVOLVED IN TRIACYLGLYCEROL BIOSYNTHESIS IN DEVELOPING SEEDS OF *CAMELINA SATIVA*

Abdullah, H. and Dhankher, O.P.
Stockbridge School of Agriculture, University of Massachusetts Amherst

Triacylglycerol (TAG) is a neutral lipid with considerable importance for dietetic, technical, cosmetics and many other industrial applications. The function and value of different plant oils is derived from the fatty acid composition of TAGs. Severe genes that regulate the fatty acids and TAG biosynthesis have been identified in Arabidopsis. However, it remains poorly understood as to how these genes contribute to the overall accumulation of TAG in the seed oil and what are the rate limiting steps in the biosynthesis of these molecules in the time course of seed development. Among many plant species that being addressed in recent literature for TAG formation/accumulation in seed oil, *Camelina sativa* has attracted much interest due to its unique oil quality and oil yield as well as the positive agronomic attributes. However, the high percentage of polyunsaturated fatty acid a-linolenic acid C18 : 3 (30-35%) and linoleic acid C18:2 (20-25%) in Camelina oil makes it highly prone to oxidation and suboptimal for biofuel and bio-based lubricant applications. One approach to increase the Camelina oil content and to alter its oil composition is to understand the transcriptome of Camelina in developing seeds that coincide with the biosynthesis and accumulation of TAG. This approach will help to investigate the gene expression changes contributed to the consequence changes in metabolite intermediates in TAG biosynthesis. Interestingly, none of the genes associated with TAG formation in oilseed of Camelina have been characterized. Therefore, to identify and characterize genes associated with the triacylglycerol biosynthesis and accumulation in Camelina developing seeds, we investigated temporal expression profiles of seven genes involved in different steps of the TAG biosynthesis within developing seeds using quantitative real-time PCR. Camelina seed-pods at four stages of seed development; 7, 14, 21, and 28 days after flowering were harvested and then RNAs and cDNAs were prepared and used as templates for qPCR analysis. Concomitantly some genes increased their expression levels in developing seeds compared to their expression in leaf and flower-buds, but showed various temporal and differential expression patterns in developing seeds. This study provided not only the initial information on promoter activity and tissue-specificity, but also showed the patterns of gene expression and regulation. Further, we are using the RNA-Seq approach to study the global seed transcriptome to identify the key rate limiting steps in TAG biosynthesis. These approaches will be helpful to understand the molecular basis of oil synthesis, identifying the rate-limiting genes during seed development that could be good candidates to create improved varieties producing better qualities in oil yield and composition.

P2

IDENTIFICATION OF PALMITOYLTRANSFERASE MUTANTS IN *ARABIDOPSIS THALIANA*

Hoskin, J. and Hrabak, E.
Department of Molecular, Cellular, & Biomedical Sciences, University of New Hampshire

Arabidopsis thaliana is a small flowering plant often used as a research model because of its short lifecycle, prolific seed production and ease of growth. This research focuses on a family of 24 genes in *A. thaliana* that encode protein S-acyl transferases (PAT). PATs catalyze palmitoylation, the thiol esterification of a C16:0 fatty acid to a cysteine residue in a substrate protein. Palmitoylation of substrate proteins can affect their membrane association, trafficking, stability and protein-protein interactions. Plants with mutations in PAT genes will be important tools in understanding the role of PATs in plant growth and development. Homozygous PAT mutant lines of *A. thaliana* were identified using PCR genotyping followed by gel electrophoresis. Once a homozygous mutant is confirmed, the region around the mutation site is sequenced to identify the exact position of the mutation within the gene. So far, 9 mutant alleles of 3 different PAT genes have been confirmed to be homozygous and the sequence of the mutated gene has been determined. Currently RNA is being isolated from each of these mutants. The mRNA will be reverse transcribed into cDNA and analyzed by PCR to see if transcript can be detected. If the knockout hypothesis is true, then no transcript will be detected, confirming these are null mutations suitable for further research.

P3

EFFECT OF GENETIC MANIPULATION ON POLYAMINES BIOSYNTHETIC PATHWAY (GLUTAMATE-ORNITHINE-ARGININE/PROLINE) IN *ARABIDOPSIS THALIANA*

Barchi, B, Turlapati, S.A., Shao, L., Majumdar, R. and Minocha, S.
Department of Biological Sciences, University of New Hampshire

We studied the effect of putrescine over-production on the expression of genes involved in the polyamine and amino acid biosynthetic pathway with a focus on the Glu→Orn →Arg/Pro node, *Arabidopsis thaliana* transgenic seedlings expressing a mouse ornithine decarboxylase gene (m*ODC*) controlled by an estradiol-inducible promoter were used. All these compounds play important roles in the overall N metabolism in plants. Transgenic seedlings produced ~40-fold more putrescine on induction vs. the wild type seedlings, with little or no effect on spermidine or spermine. Using RT-qPCR, we examined the expression of genes involved in the Glu→Orn →Arg/Pro pathway. All enzymes of the pathway except N-acetyl-Glu synthase (NAGS) are encoded by a single copy of the gene, NAGS is encoded by two copies. N2-Acetylornithine: glutamate acetyltransferase, NAGS2, and N2-acetylorrnithine aminotransferase showed very low expression in the control and the mODC-induced transgenic seedlings. Ornithine aminotransferase and ornithine transcarbamoylase showed no change in expression among all samples. N-Acetylglutamate synthase1 showed 2 fold higher expression in 24h induced mODC seedlings while the expression decreased to about half by 48h. Whereas there was no change in the N-Acetyl-Glu kinase expression in the control at 24h, mODC 24h and 48h induced samples showed 4 fold increase in expression. Carbamoylphosphate synthetase large subunit, argininosuccinate lyase, and arginase showed 2 to 5 fold increase in 24h induced seedlings vs. the control. These data suggest that high putrescine has an effect on the expression of some of the genes related to Glu→Orn →Arg/Pro pathway while others display little or no change.

P4

MANIPULATING POLYAMINE BIOSYNTHESIS TO INCREASE CARBON SEQUESTRATION IN *DUNALIELLA* SPP.

Melloni J. and Minocha S.
Department of Biological Sciences, University of New Hampshire

Biofuel produced from algae is a promising source of renewable energy. Algae biomass from the salt tolerant, thin walled, green algae of the Dunaliella is currently of interest as a potential source of biofuel. However, its efficiency for biofuel production needs to first be improved by increasing its capacity to sequester carbon and nitrogen into biomass production, in this respect a group of metabolites called polyamines can help. They are nitrogen rich molecules present in all living organisms. By increasing the biosynthesis of putrescine (a diamine - the smallest polyamine) in Dunaliella through genetic engineering we can increase the rate of carbon fixation and accumulation. However, currently no reliable system is available for genetic engineering of Dunaliella. My thesis project has two objectives: (1) to develop a reliable protocol for the transformation of Dunaliella, and (2) to manipulate the polyamine (putrescine) pathway in Dunaliella to establish if it will increase total nitrogen and carbon assimilation. To achieve this we will improve the transformation efficiency of two techniques, electroporation and biolistic bombardment, also known as the gene gun. The availability of a reliable transformation system would also make these algae desirable for use in the production of pharmaceutically important proteins on large scale. In addition we will transform the algae with genes to increase the rate of putrescine biosynthesis. By increasing putrescine biosynthesis we aim to increase the rate of carbon fixation and accumulation through which we expect to increase the biomass available for biofuel use.

P5

IDENTIFICATION OF ENHANCERS AND SUPPRESSORS OF *gravitropism defective 2* (*grv2*)

Famiglietti, K.M., Duncan, L.H., Prabhu, R. and Silady, R.A.
Biology Department, Southern Connecticut State University

GRV2 functions in vesicle trafficking, from the pre-vacuolar compartment to the vacuole (Silady *et al.,* 2008). *Arabidopsis thaliana* plants with mutations in *grv2* exhibit a number of phenotypes, all dealing with defects in asymmetrical growth and vesicular trafficking. The first observable phenotype of *grv2* mutants is seen in the two-cell stage of the plant embryo. The *grv2* mutants, unlike wildtype, have an enlarged and highly vacuolated cell at the embryo apex (Silady *et al.,* 2008). While many mutants exhibiting defects early in embryo development are embryo lethal, *grv2* mutants grow and develop into mature plants capable of producing viable seeds. Therefore, the *Arabidopsis thaliana grv2* mutant is an ideal background for a second site genetic screen.

This study aims to identify a group of mutants, each with the *grv2-1* mutation and one additional unique mutation. The additional mutations in each individual will either enhance or suppress the phenotypes caused by the original *grv2* mutation. The phenotypes that will be screened include defects in apical hook maintenance, gravitropism, phototropism, and seedling arrest in the absence of sucrose.

Silady, R., Ehrhardt, D., Jackson, K., Faulkner, C., Oparka, K., Somerville, C. (2008) The GRV2/RME-8 protein of Arabidopsis functions in the late endocytic pathway and is required for vacuolar membrane flow. The Plant Journal 53:29-41.

P6

CANDIDATE GENE DISCOVERY FOR PRIMARY REGULATORY GENES OF SOYBEAN SENESCENCE USING A MAP BASED APPROACH

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Plant senescence is a genetically determined developmental program characterized by systematic degradative processes that involves activation of new gene activity and down-regulation of other genes that ultimately leads to cell, tissue, organ and whole plant death. Elucidating senescence regulatory pathways and participating genes will allow for the development of strategies to improve crop yields and also curtail post-harvest losses. Three genes are known to be primary regulators of senescence in soybean; namely, *g, D1,* and *D2*. In double and triple mutant combinations these genes confer an evergreen leaf and seed phenotype. The double mutation *d1d1d2d2* shows an inhibition of degradation of chlorophyll and chlorophyll binding protein, but photosynthesis declines and the leaves still abscise. In the triple mutant *GGd1d1d2d2* the leaves maintain the normal photosynthetic capacity, but still abscise. So, while the senescence program is not entirely blocked in the mutant background, it is altered. Studies have shown that the expression patterns of soybean senescence associated genes (SAGs) are regulated differentially by *g*, *D1* and *D2*. Due to the pivotal regulatory nature of these three genes for senescence, it is important to identify their specific nature. As such, an analysis using available soybean genome resources (Soybase, Phytozome, COGE, etc.) was undertaken. This has resulted in the identification of a gene, Glyma01g41610.2, which encodes a putative transcription factor residing within the marker boundaries of the D1 locus on chromosome 1 which also shares a high level of synteny with a region on chromosome 11 and includes a paralogous gene, Glyma11g03770.2, within the D2 marker boundaries. Similarly, another gene, Glyma01g00510.1 also encodes a putative transcription factor and is located within the marker boundaries of the G locus on chromosome 1. These genes were selected as candidates representing *g*, *D1*, and *D2* for RT-PCR analysis. None of the initial candidates exhibited a differential expression profile when comparing wild-type and mutant allelic versions in isogenic genetic backgrounds. The *D2* and *g* genes were then selected for Sanger sequencing to determine if sequence differences were responsible for the observed phenotypic variations. No differences in sequence were observed when comparing wild type and mutant allelic forms. However, sequences variations were observed when comparing *g* in the Harosoy and the Williams 82 variety. A second candidate *g* gene (Glyma01g00520.4) was selected from the defined marker boundary interval on chromosome 1 but did not demonstrate a differential pattern of expression using RT-PCR.

Acknowledgements: RI-EPSCoR and the College of the Environment and Life Sciences at URI.

P7

DEVELOPMENTAL AND MORPHOLOGICAL CHANGES IN *NEPENTHES* PERISTOMAL NECTARY GLANDS

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The *Nepenthes* genus includes 118 species of carnivorous pitcher plants that are native to the tropical climates of southeastern Asia. *Nepenthes* acquire supplemental mineral nutrition by ingesting insects passively in modified epiascidiate leaves that form their pitcher shaped structure. Pitchers are divided into three visible zones: an "attractive zone" containing the lid, and a ridged and double-edged lip called the peristome; a "conductive zone", the upper inside surface of the pitcher with slippery epicuticular waxes; and a "digestive zone" in the base of the pitcher with digestive glands. In the attractive zone, nectar is typically excreted by extrafloral nectary glands located on the tendril, the outside of the pitcher, the pitcher lid, and the peristome. Arthropod prey is attracted to the pitchers through optical cues as well as the secretion of sweet smelling nectar.

An SEM analysis of seven *Nepenthes* species including: *N. truncata, N. maxima, N. ventricosa, N. rafflesiana, N. hirsuta, N. ephippiata,* and *N. albomarginata* showed substantial variation among three key aspects in the attractive zone that help capture and retain arthropod prey. After entering the pitcher, the arthropod escape is obstructed by the inner protruding lip of the peristome. When wet, the overlapping epidermal peristome cells create a slippery surface that interferes with the adherence of insect feet, directing the insect towards the inner lumen of the pitcher. Below the peristome is an internal, slippery wax layer that also reduces the insect's ability to climb out of the pitcher. Preliminary results indicate the nectar of *N. alata* is composed primarily of asparagine, alanine, lysine and arginine.

To better understand the developmental process of nectary glands and the method of intercellular nectar transport, we performed a TEM comparative age survey of nectary glands from three different maturation ages of *N. alata* pitchers. All cells had numerous mitochondria, ER, Golgi, plastids, and vacuoles in a dense cytoplasm as well as plasmodesmata between the individual nectary parenchyma cells. The larger class of immature pitchers (approx. 4 cm in diameter, lid closed) had budding structures on the inner cell membrane of the nectary parenchyma cells, suggesting that secondary cell wall protrusions begin their development at this age. Older mature pitchers (approx. 15 cm diameter with an open lid) had nectary parenchyma cells with full-sized cell wall protrusions, as well as numerous plasmodesmata. It remains unclear whether the nectary gland is responsible for producing the carbohydrates used to produce the nectar, the carbohydrates are imported from other parts of the plant via the vascular tissue, or if the system is a combination of both of these scenarios.

Acknowledgements: The authors acknowledge the contributions of Hope Barone, Jacquelyn Durand, Jeffery Sumner, Daniel Smoot, Cara Hass, and Lesley Sutherland.

P8

ANALYSIS OF DAILY UBIQUITYLOME DYNAMICS TO DETERMINE CIRCADIAN CONTROL OF GLOBAL PROTEIN DEGRADATION

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The endogenous timekeeping mechanism, known as the circadian clock, is inherent to all forms of eukaryotic life. From the circadian clock, organisms receive a fitness advantage through the ability to coordinate metabolic processes with environmental circumstances. Environmental cues such as light and temperature entrain the circadian clock, allowing for optimized daily phenotype patterns. In *Arabidopsis thaliana*, genes associated with photosynthesis, hypocotyl elongation, timing of flowering, and resistance to cold all display circadian influence.

In understanding the internal driving mechanisms of the circadian clock, much work has been done on the transcriptional and translational details of clock mechanisms. As a result, the plant community now enjoys a relatively robust understanding of the transcriptional network that composes the core of clock function. However, much less is known about the circadian nature of post-translational modification and degradation of protein and how this influences oscillations in protein abundance. The project presented in this poster aims to lessen this dearth of understanding and to produce a deeper understanding of clock controlled protein degradation, which is critical for clock function and connectivity to vital output processes.

By using improved mass spectrometry techniques, and by enrichment of ubiquitylated proteins by immunoprecipitation, we aim to search the entire proteome of *A. thaliana* for proteins that exhibit a circadian oscillation in their ubiquitylation profiles. By understanding when certain clock proteins are targeted by the ubiquitin proteasome system, we will gain valuable insight into the circadian clock's influence on the entire life cycle of a protein. The presented poster will include preliminary data of the strength and functionality of our approach, as well as a detailed experimental design. It will also demonstrate the power of peptide-level ubiquitylome studies.

P9

TARGETS OF CLOCK PROTEINS FKF1 AND LKP2: A FIRST GLANCE

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In plants, the circadian clock is essential for properly regulating the timing of many growth events, including hypocotyl elongation and flowering time. Traditional approaches for studying clock-dependent gene expression misrepresent a significant portion of the picture, however, as translational and post-translational regulation can lead to strongly cycling protein abundance from a non-cycling transcript, or steady protein levels from a robustly cycling transcript. Standard proteomics approaches are not particularly effective, as many of the clock proteins are unstable or of too low abundance to detect in whole proteome approaches. In this study we demonstrate the preliminary results from a modified proteomics approach, in which the F-box domain is replaced with a HIS-FLAG affinity tag in FKF1 and LKP2, two *Arabidopsis thaliana* clock F-box-containing E3 ubiquitin ligase substrate adaptors. As the F-box domain confers the inherent instability into the protein and its binding partners by bridging interaction with the SKP-CULLIN ubiquitin ligases, its removal allows substrate stabilization rather than destabilization. Affinity tags can then be used for immunoprecipitation followed by mass spectrometry, simplifying the previously difficult task of identification of these proteins' target substrates.

P10

LINKING CA2+ TO THE BRASSINOSTERIOD SIGNAL TRANSDUCTION CASCADE: MOLECULAR STEPS THAT MODULATE BR RESPONSIVE GENE EPXRESSION

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Brassinosteroid (BR) hormones bind to the receptor BRI1 (BRASSINOSTEROID INSENSITIVE 1) and control growth and development through a phosphorylation/dephosphorylation signaling cascade, which mediates downstream gene regulation. Previous work from our lab indicates that cytosolic Ca^{2+} elevation is involved in the BR signal transduction cascade. A recent report shows that in the presence of Ca^{2+}, Calmodulin (CaM) 7 binds to the BRI1 cytoplasmic domain (Oh et al., 2012, Biochem J. 443:515), consistent with Ca^{2+} involvement in BR signaling. CaM-binding transcription activators (CAMTAs) are activated by CaM binding and facilitate Ca^{2+}-dependent gene expression. Previous work in our lab shows INDOLE-3-ACETIC ACID-INDUCIBLE1 (*IAA1*) and PHYTOCHROME B ACTIVATION-TAGGED SUPPRESSOR1 (*BAS1*) are two genes up-regulated by BR in a Ca^{2+}-dependent pathway. Here qPCR shows BR no longer has an effect on *IAA1* and *BAS1* in Arabidopsis *camta3-1* mutant seedlings. BR-dependent expression of SMALL AUXIN UP RNA1 FROM ARABIDOPSIS THALIANA ECOTYPE COLUMBIA *(SAUR-AC1)*, a gene known to respond to BR directly through the phosphorelay cascade, is not impaired in the *camta3-1* mutant. Treatment of wild type seedlings with a CaM antagonist W7 prior to BR addition impaired *IAA1* and *BAS1* expression but did not affect expression of *SAUR-AC1* the effects of the CaM antagonist were consistent with the *camta3-1* results. Exogenous BR causes a de-etiolated hypocotyl phenotype in the dark. Our results show that BR no longer impairs the hypocotyl length in *camta3-1* seedlings. These results suggest that *CAMTA3* may decode BR-induced Ca^{2+} elevations and transmit this signal to the nucleus to modulate the expression of at least some (*IAA1*, *BAS1* but not *SAUR-AC1*) BR responsive genes and control some BR-dependent phenotypes. This work further characterizes the generation of a Ca^{2+} signal, and the transmission of this signal to the nucleus as steps in at least one component of the BR signal transduction cascade.

P11

IDENTIFYING MUTATION SITES IN PROTEIN PHOSPHATASE 2A CAUSING SODIUM-DEPENDENT ROOT SKEWING IN *ARABIDOPSIS THALIANA*

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Multiple genes and pathways are involved in directing growth and stress responses in plant roots. For example, under salt stress, protein phosphatase 2A (PP2A) is critical for maintaining root structure. Mutations in several of the genes encoding subunits of PP2A cause a sodium-dependent root skewing phenotype. To further understand this signaling pathway, more mutants need to be identified. Publicly available collections of *Arabidopsis thaliana* T-DNA mutants were used to screen for new mutants with a sodium-induced root skewing phenotype. TAIL-PCR followed by DNA sequencing was used to identify the mutation site in the *A. thaliana* genome. The locations of T-DNAs in three *srs* mutants have been found so far. The *srs-1* mutation appears to be in the inter-genic region between genes encoding phosphatidylinositol-4-phosphate 5-kinase and the U2 snRNP auxiliary factor, a T-DNA in *srs-2* is located between genes *JAC2* and *RRA2* and in *srs*-3 between a pseudogene and a gene with an unknown function. Progress toward determining the T-DNA insertion site of the other mutants identified in the screen will be reported.

P12

EXAMINATION OF THE THREE PUTATIVE FUNCTIONAL RESIDUES IN THE PUTATIVE GUANYLYL CYCLASE DOMAIN IN PEPR1 AND BRI1

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Like in animals, cGMP or cAMP play important roles in signaling transduction in plants. However, unlike in animals, little is known about guanylyl cyclase (GC) in plants and no GC mutant has been identified and characterized. Bioinformatics searching revealed several receptor proteins that may contain GC activities, including PEPR1 and BRI1. Recent studies showed Increase of fluorescence in roots of FlincG (Fluorescence indicator of cGMP) plants treated with either Pep or BR, suggesting that the guanylyl cyclase domain located in the C-terminus of PEPR1 and BRI1 could be responsible for making cGMP in Arabidopsis. By comparing with animal GC, three amino acids in the domain, which are conserved among these receptors, were identified to be putative functional residues. Based on this information, we made mutations of these three residues and transformed the mutated proteins back into the corresponding mutant plants. The transgenic plants expressing $BRI1^{m}$ showed intermediate phenotype between wild type and *bri1-5* mutant plants. Gene expression analysis showed that BR induced transcripts, such as *IAA1* and *SAUR-AC1*, were reduced in transgenic plants compared to wild type. However no significant difference between transgenic plants and mutant plants were detected. These results indicate that these three residues are important for BRI1 function.

The *pepr1* mutants expressing aequorin were transformed with $PEPR1^{m}$. The transgenic plants showed similar Ca^{2+} increase compared to wild type after Pep induction. Gene expression analysis also showed no impairment of *MPK3* and *WRKY33* transcripts in transgenic plants. These results are opposite to what we observed in BRI1 transgenic plants. This might be due to the different functions of these residues in different signaling pathways or the different promoters used in the transgene constructs. Kinase activities of the mutated proteins are under investigation.

Acknowledgements: This work is supported by National Science Foundation (award no. 1146827 to G.A.B.).

P13

ONE-STEP MICROPROPAGATION FROM LEAFY TISSUE INTO ROOTED PLANTLETS IN A MIST BIOREACTOR

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Micropropagation is challenging to automate mainly because of the diverse morphology of plant shoots. We have shown that the mist reactor can be used for 1-step propagation of carrot cells into embryos and then fully rooted plantlets using poly-L-lysine (PLL) coated polypropylene or nylon strips to which cells were attached prior to growth while hanging in the nutrient mist. It is also possible to propagate small attached explants of leaves since *Artemisia annua* leaf explants, for example, attached by their filamentous trichomes to PLL coated polypropylene or nylon. Before bridging otherwise discrete micropropagation steps together into 1-batch culture, we would like to know how explants respond to mist reactor conditions, i.e. ventilation, misting cycle, and light intensity during shooting, rooting and acclimatization, respectively. For shooting, *in vitro A. annua* cultures were chopped into 4 types of inoculums, leaf only (L), leaf with petiole (LP), node (N) and internode (IN), and then cultured for 2 weeks with shooting medium in mist reactors and corresponding modified Magenta box controls under ventilation and light conditions used in the reactors. In all conditions, reactor cultures yielded more biomass, taller new shoots with more shoot apical meristems (ShAM) and new shoots (SH) per new shoot generating explant compared to their Magenta controls. Among all 4 types of inoculums, N always had highest shooting percentage (>80%) and tallest new shoots regardless of culture conditions. Overall shooting percentage was not different between reactor and Magenta control under all conditions except with 0.1 vvm CO_2 enriched air ventilation; these reactors had higher shooting percentage than their Magenta controls (25% VS 14%). Shooting percentage of LP and IN was higher in reactor than Magenta controls under CO_2 enriched and 0 vvm conditions. However, hyperhydricity was greater in reactors than Magenta controls. CO_2 enriched reactors yielded more biomass, greater shooting percentage (i.e. for LP), taller new shoots with more ShAM and SH/plant, and less hyperhydricity than 0 vvm and 0.1 vvm ambient air ventilated reactors. When light intensity was increased in CO_2 enriched reactors, biomass increased and hyperhydricity declined further than cultures grown in CO_2-enrichment-only reactors. Shooting in Magenta controls was no different across all conditions except that CO_2 enrichment seemed to yield more SH/plant and SH/Sham compared to 0 vvm and 0.1 vvm ambient air conditions. Hyperhydric shoots generally had fewer ShAM and SH/plant, and SH/ShAM, greater % water content, and were taller than normal shoots. Compared to normal shoots, hyperhydric leaves also had fewer glandular trichomes. Both the mist reactor and our attachment technology seem to offer opportunities for at least partial automation of micropropagation

P14

IN SITU EMBRYO RESCUE AS A NOVEL METHOD FOR RECOVERY OF NON-GMO HYBRIDS FROM WIDE CROSSES

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Rapid genetic improvement of various crop species are anticipated by current advances in genomics, bioinformatics, association genetics, marker assisted breeding, conventional genetics and other non-GMO approaches. While transgenics offer access to traits outside the conventional breeding pool they are time consuming, costly, and involve unresolved issues regarding gene confinement, USDA deregulation and commercial release. Wide crosses have been used as a method in plant breeding for decades and proven to be a useful method for transferring novel genetic materials and traits for new cultivar development. Historically many products of wide crosses require tedious and inefficient embryo rescue prior to embryo abortion to recover plants that are often sterile due to genetic incompatibility. We have utilized transgenic switchgrass (*Panicum virgatum*, cv *'Alamo'*) and herbicide or antibiotic selection for recovery of wide intra-and inter-specific F1 crosses by a novel method we have termed *'in situ embryo rescue'*. Hybrids were generated between transgenic switchgrass lines and wild type Atlantic Coastal Panicgrass (*Panicum aramrum* Ell. var. *amarulum*). In one set of crosses, the male parent, a transgenic *Alamo* line (*HYG* resistant, and *GFP* positive) was used as a pollen donor with the female WT ACP plants. The selection procedure was used to select F_1 embryogenic callus from immature caryopses, and clonal plants were successfully regenerated. Using a similar procedure with herbicide resistance in place of *hyg*, a subset of the F_1 hybrid plants were backcrossed to WT reference *Alamo* and non-GMO F_1BC_1 progeny were recovered by screening for herbicide sensitivity using the "leaf painting" assay (3% Finale). The opportunity presented here involves the development of an innovative breeding strategy that makes use of transgenic herbicide resistance for early embryo rescue from wide crosses as genetic bridge intermediates followed by backcrossing to recover non-GMO hybrids that it is likely applicable to other crop species.

P15

DISTRIBUTION OF L-DOPA IN DIFFERENT PARTS OF FAVA BEANS

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L-Dopa has been shown to be an effective drug for the treatment of Parkinson's disease. Synthetic L-Dopa is relatively expensive and some side-effects have been reported that are associated with it. Fava Bean has been reported as one of the nature's resources of L-Dopa and clinical studies have shown that its anti-Parkinson's effects have no or minimal side-effects compared with synthetic forms. The accumulation of L-Dopa in different organs of fava beans was studied in a field grown study in Pioneer Valley in Massachusetts. Windsor which is currently is the common fava beans variety in New England was planted on April 12013 at UMass Research and Education Center. Fava beans were harvested at two stages of growth. The first harvest was at six-leaf stage and the second harvest was when pods were fully grown. For L-Dopa determination in first harvest the whole seedling was used. However, when pods were ready to harvest the whole plants were diged out and divided into various parts including roots, stems, leaves, terminal buds and immature seeds. All organs were digested separately and analyzed using HPLC. The results showed that seedling stage had the highest amount of L-Dopa (13.3 mg g^{-1} plant dry weight. Fava beans stem had the least amount 3 mg g^{-1}.

P16

PRODUCTION OF DECAFFIENATED TEA THROUGH GENETIC ENGINEERING

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With the recent escalation in concern about the adverse effects of caffeine on human health, there is a market for decaffeinated tea. Current industrial techniques used to extract caffeine result in tea lacking in flavor and nutrients. By genetically modifying the synthesis of caffeine in the common tea plant, *Camellia sinensis*, caffeine production can be turned off (or reduced) and a naturally-decaffeinated product can be produced. Caffeine synthase (CS) is the primary enzyme responsible for caffeine biosynthesis in tea. The gene responsible for coding this enzyme has been previously cloned by the Minocha Lab at the University of New Hampshire. The CS gene has been further modified by an undergraduate student in the lab to make it ready for transfer into *Agrobacterium tumefaciens*. Moving forward, it is necessary to ensure the functionality of the gene by adding it to a bacterial expression vector. Once confirmed, an antisense version of the gene can be made. The transcript (RNA) of the antisense gene when combined with the wild type CS mRNA in the plant, will lead to the latter's degradation, thus inhibiting the production of caffeine in the cells. This antisense version of the gene will then be transferred into the plant cells, which will be grown into transgenic plants that will later be tested for caffeine production.

Acknowledgements: Dr. Subhash Minocha, Professor of Plant Biology at the University of New Hampshire.

P17

EFFECT OF ROOTS ON ARTEMISININ AND FLAVONOID PRODUCTION IN SHOOTS OF *ARTEMISIA ANNUA*

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Artemisinin is a potent antimalarial sesquiterpene lactone produced and stored in the glandular trichomes of *Artemisia annua* L. Although they produce no artemisinin, nor any of the precursor compounds, *A. annua* roots appear to have a regulatory effect on production of the terpene in leaves. However, more information is required to clearly define the role of the roots on artemisinin production in the plant. Two experimental approaches were used. First, *A. annua* plants were elicited with chitosan or salicylic acid applied either as a foliar spray or delivered to the roots. Both the tips of the apical meristem and the ninth through twelve leaves from the tips were harvested at 48 and 96 hours, and 96 and 120 hours, respectively for plants treated with chitosan or salicylic acid. Artemisinin and flavonoids that reportedly act synergistically with the drug were extracted and quantified using GC/MS and an aluminum chloride assay, respectively. Second, micrografting was performed between high and low or null producing *A. annua* clones. In contrast to other reports, results thus far showed that foliar elicitation with chitosan and salicylic acid had no effect on flavonoid production in the shoots. Root elicitation comparisons are in progress as is the analysis of artemisinin in elicited plants. Preliminary grafting results, however, showed that the rootstock of high producing *A. annua* increased the production of artemisinin in the scion of low producing *A. annua*. This study will provide valuable information regarding the role that roots play in the production of artemisinin in the shoots.

Acknowledgements: This project was partially supported by Award Number NIH-2R15GM069562-03 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

P18

INVESTIGATING THE LIMITING STEP IN THE TRANSCRIPTION OF THE VINDOLINE PATHWAY IN *CATHARANTHUS ROSEUS* USING RT-PCR.

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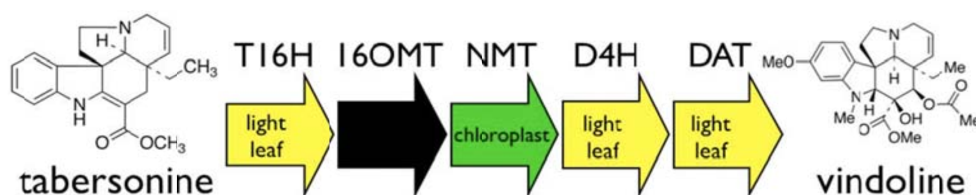
Catharanthus roseus is a widely studied plant, as it is the natural source of terpenoid indole alkaloids (TIAs), a class of medicinal compounds that include the valuable chemotherapeutics vinblastine (VBL) and vincristine (VCR). These important compounds are naturally found at very low concentrations in the plant, and production difficulties contribute to high drug costs of $4-40 MM/kg. The complex TIAs cannot be organically synthesized, and the estimated 30 enzymatic steps for its biosynthesis cannot be expressed recombinantly in a bacterial host. Therefore, manipulating the native regulatory pathway to increase TIA concentration in the plant may be the most promising means to reduce production costs of VBL and VCR.

The work presented here focuses on the production of vindoline, a direct and limiting (~0.00003% by dry weight) precursor of VBL and VCR. Limited production of vindoline (and therefore VCR and VBL) in plant tissue cultures is believed to be related to the absent or low expression of specific enzymatic proteins in the pathway that converts tabersonine to vindoline. Some transcripts have been localized to certain leaf cell types, and some proteins are activated by light.



PCR techniques were used to investigate the expression of this pathway in whole plants, hairy root cultures, and callus cultures to better understand its tissue-specific regulation. While callus cultures (two lines) did not show any consistent patterns in expression, hairy root cultures elicited with 250 uM methyl jasmonate did show expression of all genes involved in the tabersonine to vindoline conversion. Although some transcripts are still likely at extremely low levels, this makes hairy root cultures a promising target for genetic engineering targets to increase vindoline production.

Future work will involve quantifying expression levels in the vindoline pathway via qRT-PCR to determine the limiting step(s) in the conversion. Relative comparisons between different tissues with varying levels of differentiation (calli, hairy roots, seedlings) and growth conditions will help to better understand the factors that affect the complex regulation of vindoline biosynthesis.

P19

PROMOTER ANALYSIS OF *ZCT1*, A KEY REPRESSOR OF ALKALOID PRODUCTION IN *C. ROSEUS*

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Due to their sessile existence, plants rely on rapid, well-adapted responses to environmental stresses. A common response to these stressors is the activation of secondary metabolism and the production of defense compounds. *Catharanthus roseus* provides a useful model for studying secondary defense pathways as it produces numerous Terpenoid Indole Alkaloids (TIAs) in response to certain environmental stressors, such as wounding or the presence of a pathogen. Furthermore, several TIAs are pharmaceutically valuable, including the anticancer drugs vincristine and vinblastine. However, these drugs are very expensive because of their low concentrations in the plant.

Over the last decade, there has been remarkable advancement in the understanding of TIA biosynthesis, including the sequencing of biosynthetic enzymes, discovery of transcription factors regulating biosynthesis, and elucidation of the pathway induced by the plant hormone Jasmonate (JA). JA has been shown to initiate TIA production by inducing expression of a network of transcription factors, including the activator ORCA and repressor ZCT.

Here, we report the sequencing and analysis of the *Zct1* promoter, a key transcriptional repressor of TIA biosynthesis. Approximately two kilobases upstream of the *Zct1* coding sequence was obtained using a genome walking approach. This promoter region was analyzed *in silico* using the Plant Cis-Acting Regulatory Element (PlantCARE) database. Several putative regulatory elements were identified, and their functionality is being tested via both *in vitro* and *in vivo* techniques.

Acknowledgements: NSF – CBET Award Number 1033889, members of the Cram and Lee-Parsons Labs, and Northeastern University's Departments of Biology and Chemical Engineering.

P20

ACCELERATING BIOENERGY: THE EFFECT OF NPK FERTILIZER REGIMES ON THE RECOVERY OF TRANSPLANTED CLONES OF SWITCHGRASS (*PANICUM VIRGATUM* CV ALAMO) ATLANTIC COASTAL PANIC GRASS (*PANICUM AMARUM),* AND GENETICALLY MODIFIED SWITCHGRASS (*PANICUM VIRGATUM* CV ALAMO).

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Switchgrass and related species are important bioenergy crops. Fertilizers, specifically containing nitrogen, phosphorus, and potassium (NPK), are essential to plant growth and biomass yield. This study measured the biomass of transplanted clones of wild type switchgrass *(Panicum virgatum* cv Alamo*)*, Atlantic Coastal Panic Grass *(Panicum amarum;* ACP*)*, and switchgrass which was genetically modified with genes unrelated to biomass production. The central goals of this study were to compare effects of NPK ratios on biomass yield, evaluate if genetic modification unto itself would affect biomass production, and assess a novel method for measuring biomass using non-destructive photographic green pixilation. Seven fertilizer regimes were given to plants cut to 10 inches. After two months, biomass was evaluated through green pixilation and qualitative observation. This was the first application of pixilation on individual plants as a measure of biomass; the method was further improved. Results show that application of high phosphate fertilizer, 10-52-10 NPK, resulted in highest biomass amounts. Wild type and genetically modified switchgrass had similar pixilation values (and, thus, biomass), with both grasses having higher values than ACP. Quantitative green pixilation showed comparable results to qualitative observation. In conclusion, transplanted clones of switchgrass and ACP increase biomass in response to high phosphate fertilizers when compared with other NPK ratios. The process of genetic modification with genes unrelated to biomass production did not unto itself affect yield. Green pixilation has significant potential as a new method to quantitatively measure biomass. Fertilizer regimes are important contributors to biomass yields for these bioenergy crops.

P21

DEVELOPMENT OF GENETIC ENGINEERING TECHNIQUES IN MICROALGAE FOR BIOFUEL PRODUCTION

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Fossil fuel is unsustainable and has contributed to pollution as well as global climate change creating an urgent need for alternative, sustainable fuels. Biofuel produced from fast-growing, oleaginous (high oil-producing) microalgae such as *Chlorella vulgaris*, has the potential to be a cost-effective and sustainable alternative to fossil fuel. Although there is potential to produce renewable energy using microalgae, economically feasible large-scale production of microalgal biofuel has been hampered by costs associated with the harvest, extraction, and downstream processing of lipids. One method for counteracting these costs is to genetically engineer microalgae to increase biofuel production rate. Although there has been recent progress in genetically engineering microalgae, development of high oil-producing transgenic lines has been impeded by a lack of information on TAG biosynthesis in microalgae. Here we describe optimization of a transient expression system in *C. vulgaris* using *Agrobacteria* (a plant pathogen capable of inserting genes into the host genome). We show that *Agrobacterium* strain, culture age, culture density, and co-cultivation duration are important variables for improving transformation efficiency. This optimized method can be used to achieve transgene expression in >70% of the transformed microalgae. This will enable optimization of overexpression constructs as well as identification of key lipid biosynthetic genes that can be targeted to engineer improved microalgae strains for biofuel production**.**

Acknowledgements: This work was supported in part by the Catalyst Grant from the Massachusetts Clean Energy Center to C. Lee-Parsons. Construction and cloning of plasmids used in this research was done in part by Jessica Weaver in the Biology Department at Northeastern University.

P22

RECOVERY OF INTRASPECIFIC AND INTERSPECIFIC HYBRIDS IN SWITCHGRASS (PANICUM VIRGATUM L.) VIA A TRANSGENIC HERBICIDE RESISTANCE SELECTABLE MARKER

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Rapid genetic improvement of various crop species are anticipated by current advances in genomics, bioinformatics, association genetics, marker assisted breeding, conventional genetics and other non-GMO approaches. Here we demonstrate the use of an herbicide resistance selectable marker (bar) for recovery of intraspecific hybrid offspring of 'Alamo' switchgrass (Panicum virgatum cv. Alamo) and 'Southlow' switchgrass (Panicum virgatum cv. 'Southlow') as well as interspecific hybrid offspring of switchgrass (Panicum virgatum cv. 'Alamo') and 'Atlantic' Coastal Panicgrass (Panicum amarum Ell. var. amarulum). Pollen cages were set up enclosing a bar positive transgenic 'Alamo' switchgrass primary transformant that served as a pollen donor to either a wild-type 'Southlow' individual or a wild-type 'Atlantic' Coastal Panicgrass individual as a maternal parent. After seed maturation and germination the developing seedlings were assayed for bialaphos resistance by an application of 3% bialaphos. Resistant plantlets were verified for inheritance of the bar gene nuclear marker from the T0 'Alamo' parent through Southern blot. A cytoplasmically inherited cpDNA marker was utilized to verify the maternal inheritance of the 'Atlantic' Coastal panicgrass cytoplasm in the hybrid offspring. Bar positive hybrid offspring were backcrossed to wild-type 'Alamo' plants and bialaphos-sensitive offspring of this cross were isolated as non-transgenic novel hybrid germplasm. This platform could serve as a method for combining desirable characteristics through exploiting additive genetic variation and provide a more timely approach to developing novel switchgrass lines for increased biomass production for biofuel production.

P23

TRANSGENIC EXPRESSION OF *THERMOMYCES LANUGINOSUS* AND *CANDIDA ANTARCTICA* LIPASES IN PLANTS FOR THE ENZYMATIC PRODUCTION OF BIODIESEL

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Enzymatic transesterification with lipase as the catalyst eliminates soap formation. Unlike alkali-based reactions, the products can easily be collected and separated. Moreover, enzymes require much less alcohol to perform the reaction, and can be reused despite some loss in activity at the end of each cycle. We have genetically engineered plants to constitutively express a lipase for biodiesel production from spent oils. We have cloned the gene of a lipase with known transesterification activity from *Thermomyces lanuginosus*, and *Candida antarctica*. Cloning of TL enzyme involved isolation of total RNA, reverse transcription of the mRNA into cDNA and PCR amplification of the lipase gene using specific primers. The gene was first inserted into a cloning vector (pCR8/GW/TOPO) and sequenced to confirm its identity. The gene has been inserted into a plant destination vector (pGWB408 and pMDC83) via LR clonase reaction. *Nicotiana tabacum* (tobacco) leaf was transformed with the lipase gene using *Agrobacterium tumefaciens* (strain GV3101) and transferred onto selection media plates. We have also grown *Arabidopsis thaliana* from seeds that were transformed using the floral dip transformation method. The recombinant enzyme was collected from the genetically engineered plants, purified, and tested for both hydrolytic and synthetic activity. The activity results will be compared with enzyme catalysts from commercial *Thermomyces lanuginosus and Candida antarctica,* and the effect of solvent addition will also be presented.

P24

HIGH RESOLUTION IMAGING OF MYOSIN XI-DRIVEN VESICLES ON ACTIN FILAMENTS IN THE MOSS *PHYSCOMITRELLA PATENS*

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Land colonization, sexual reproduction and water and nutrient uptake are fundamental plant processes that rely on highly polarized cell growth, also called tip growth. This specialized form of cell growth requires both a dynamic actin cytoskeleton and active secretion of plasma membrane and cell wall components at the apex of the elongated cell. We hypothesized that the F-actin-associated motor, myosin XI, is a primary orchestrator of actin dynamics and vesicular trafficking at the cell apex. Nevertheless, the identity of the myosin XI-driven endomembrane vesicles and their mode of transport remain poorly characterized in plants. Using the tip growing cells of the moss *Physcomitrella patens*, we previously showed that myosin XI and F-actin co-localize at the cell apex, and that increases in myosin XI levels anticipate F-actin levels. In contrast, we found that myosin XI levels show an identical phase relationship with a vesicle marker (VAMP). To gain further insight into the myosin XI-mediated vesicular transport in moss cells, we used dual color Total Internal Reflection Fluorescence microscopy, which allows high resolution imaging necessary to track endomembrane vesicle motility. Consistently with our previous results, we show that myosins XI and VAMP label the same endomembrane vesicles and that a fraction of these vesicles are motile. Furthermore, we demonstrate for the first time in moss cells that myosin XI-driven vesicles are moving along actin filaments. Finally, we provide evidence that myosin XI also co-localizes with Rab proteins on endomembrane vesicles, suggesting that, similarly to their animal and yeast homologues, plant myosins XI need adaptor proteins to bind their endomembrane cargo. This approach provides a better understanding on how myosin XI coordinates the vesicular trafficking machinery and the actin dynamics to maintain polarized growth at the apex of plant tip growing cells.

P25

INVESTIGATING THE ROLE CHLOROPLAST MOVEMENTS PLAY IN NON-PHOTOCHEMICAL QUENCHING IN *ARABIDOPSIS THALIANA*

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Chloroplasts movements in Arabidopsis thaliana are stimulated by blue light and vary depending on the quantity of light. Under low light levels (<2 µmol m-2s-1), chloroplasts accumulate on the upper and lower sides of the palisade cells to maximize light absorption (accumulation response). During the high light stimulated avoidance response, chloroplasts minimize absorption and photo-damage by stacking on top of one another near the side walls of the cell. These movements alter light absorption, suggesting they would have a direct affect on photosynthesis and non-photochemical quenching. The purpose of this study was to quantify the effects that chloroplast movements have on electron transport rate, non-photochemical quenching, and photo-chemical quenching in *A. thaliana*. Plants mutated in the CHUP1 protein, known to be involved in attaching chloroplasts to myosin motors, were used as a control as they lack blue-light dependent chloroplast movements. Red light, which does not induce chloroplast movements, was also used as a photosynthetic control. Simultaneous fluorescence and percent transmittance measurements were made to compare the changes in photosynthetic parameters on the timescales of chloroplast movements. Our results indicate obvious differences in non-photochemical quenching between wild-type and CHUP1 plants. Currently, the kinetics of intensity dependent changes in transmittance are being analyzed to determine the extent to which the two phenomenon are linked.

Acknowledgments: We thank the US Department of Energy for funding this project.

P26

FUNCTIONAL ANALYSIS OF CESA STRUCTURAL FEATURES USING A GENETIC COMPLEMENTATION ASSAY IN *PHYSCOMITRELLA PATENS*

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Cellulose synthases are found in a wide range of organisms, from bacteria to land plants. However, the cellulose synthases found in land plants (CESAs) form large, multimeric, rosette-shaped cellulose synthase complexes (CSCs) and have three unique regions not found in other cellulose synthases; the N-terminal zinc-binding domain, the Plant Conserved Region (P-CR) and the Class Specific Region (CSR). The CSR, a portion of the large cytoplasmic region that contains the catalytic domain, has been predicted to be involved in CSC formation through *de novo* computational modeling. In this project, we seek to test the hypothesis that the CSR is crucial for CESA function and may play a role in CSC formation. We have developed a complementation assay in the moss *Physcomitrella patens* based on the *ppcesa5* mutant, which does not produce gametophores. In *P. patens*, CESAs in the A Clade (CESA3, 5 and 8) have similar CSR structures that are distinct from those in the B Clade (CESA4, 6, 7, 10). The *ppcesa5* mutation is complemented by overexpression of PpCESA3 and PpCESA8, but not by the overexpression of CESAs in Clade B. We have constructed an overexpression vector in which the CSR from *PpCESA5* was swapped with the CSR from *PpCESA4* and transformed it into the *ppcesa5* mutant. This construct was unable to rescue *ppcesa5* KO phenotype, indicating that the CSR is necessary for clade-specific CESA function. However, a vector in which the CSR from *PpCESA4* was swapped with the CSR of *PpCESA5* was also unable to rescue the *ppcesa5* KO phenotype. This signifies that more than the CSR is necessary for clade-specific CESA function. New studies indicate that a vector in which the C-terminal region of *PpCESA5* was swapped with the C-terminal region of *PpCESA4* was able to rescue the *ppcesa5* KO mutant. This suggests that the functionality of the C-terminal region, containing six transmembrane helices, is not clade-specific. The CSR may be interacting with an N-terminal domain, possibly the P-CR or zinc-binding domain.

Acknowledgements: This research is supported as part of The Center for LignoCellulose Structure and Formation, an Energy Frontier Research Center funded by the US Department of Energy, Office of Science, Basic Energy Sciences under award #DE-SC0001090 and is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center, which is supported in part by the National Science Foundation (MRI Grant No. DBI-0215393 and EPSCoR Grant Nos. 0554548 & EPS-1004057), the US Department of Agriculture (Grant Nos. 2002-34438-12688 and 2003-34438-13111), and the University of Rhode Island.

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LOCALIZATION OF CELL WALL POLYSACCHARIDES IN *PHYSCOMITRELLA PATENS*

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Physcomitrella patens is a bryophyte characterized by a predominantly haploid lifecycle. The haploid phase consists of protonemal filaments that enlarge by tip growth and well as leafy gametophores with several different cell types that enlarge by diffuse growth. Immunofluorescence labeling has been employed to localize some components of the cell wall, but these studies have not comprehensively examined cell wall polysaccharide composition in all cell types. We aim to obtain a more complete view of *P. patens* cell wall composition using a broader array of monoclonal antibodies and carbohydrate binding molecules (CBM) in order to provide a basis for analyzing mutant lines. Probes that detect cellulose (CBM28 and CBM3a), hemicellulose (400-2, 400-4, LM5, LM10, and LM15), pectin (LM18, LM19, LM20, LM6) and arabinogalactan proteins (JIM13) were used to stain gametophores, rhizoids and protonemal filaments of wild type *P. patens*. Strong fluorescence indicated that rhizoid cell walls are enriched in crystalline cellulose, 1, 5-α-arabinan, and xyloglucan. Medium levels of fluorescence indicated the presence of 1, 3-β-glucan, 1, 4-β-mannan, and nonesterified homogalacturonan. Lastly, there were low levels of staining for galactan, esterified homogalacturonan and amorphous cellulose, and a complete absence of staining for arabinogalactan protein and xylan. The walls of regenerated protoplasts were stained with the same antibodies appeared to be enriched in 1, 3-β-glucan, 1, 4-β-mannan, crystalline cellulose, and esterified homogalacturonan and contain medium amounts of 1, 5-α-arabinan, xylan, and xyloglucan. Low levels of staining indicated small amounts of arabinogalactan protein and galactan. There was an absence of staining for nonesterified homogalacturonan and amorphous cellulose. In gametophores homogalacturonan, 1, 5-α-arabinan, and crystalline cellulose were detected in all tissues. In contrast, 1, 4-β-galactan was localized to the conducting cells of the gametophore axis and leaves, xylan was localized to axillary hairs, and xyloglucan was localized to rhizoids. No staining of arabinogalactan protein was observed in the gametophores. The results indicate that cell wall composition varies among the different *P. patens* cell types.

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CELLULOSE UPREGULATION UNDER OSMOTIC STRESS IN *PHYSCOMITRELLA PATENS*

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Cellulose synthesis has been extensively studied in the model vascular plant *Arabidopsis thaliana*, yet many aspects of this process are not understood. Previous studies have shown that cellulose deposition decreases under osmotic stress in *Arabidopsis*. However, in *Physcomitrella patens,* cellulose deposition is dramatically increased under osmotic stress induced by supplementing the culture medium with mannitol. These opposing responses to osmotic stress may be due to differences in water uptake and drought tolerance mechanisms, given that mosses are poikilohydric and vascular plants are homeohydric. Based on public microarray data (Cuming et al., 2007, *New Phytol 176*: 275), we hypothesize that cellulose synthase genes *PpCESA*6, *PpCESA*7, and *PpCESA*8 are responsible for the upregulation of cellulose deposition in *P. patens*. Knockout (KO) mutants of *PpCESA*6, *PpCESA*7, and/or *PpCESA*8 were constructed to test the prediction that these genes are required for enhanced cellulose deposition under osmotic stress. *Ppcesa8*KO and wildtype protonemal tissues were analyzed for cellulose deposition under osmotic stress using CBM3a affinity cytochemistry. Fluorescence quantification with ImageJ showed that two *ppcesa8KO* lines have decreased cellulose deposition compared to wildtype, with P values of 0.034 and 0.033. A similar analysis comparing *Ppcesa6/7KO* and wildtype protonema showed no significant differences. The *cesa8KO* lines are also more sensitive to salt stress compared to wildtype, further suggesting a role for cellulose in stress tolerance. To localize *PpCESA* expression and confirm microarray data, *PpCESA promoter::GUS* transgenic lines were constructed. *PpCESA6pro::GUS, PpCESA7pro::GUS* and *PpCESA8pro::GUS* are expressed in the protonema. Expression levels of *PpCESAs* and PpCSLDs (cellulose like protein D) in response to osmotic stress are being examined by RT-qPCR.

Acknowledgements: This work was supported by the US Department of Energy, Office of Basic Energy Sciences as part of The Center for LignoCellulose Structure and Formation, an Energy Frontier Research Center.

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BUILDING SKILLS FOR INQUIRY IN BIOLOGY: USING MICROPIPETTERS AND STANDARD CURVES

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Practice of lab and data analysis skills in many classrooms both in secondary education and higher education settings is often neglected in the effort to address content knowledge. However, in order to allow students to begin to think like scientists, it is important to provide them opportunities to learn about the tools of the trade and how some of these tools are applied. These are the major goals of an ongoing collaboration between members of the Roberts Research Group at the University of Rhode Island and Scituate High School. This poster features two activities recently utilized as part of this project. The first is an activity to train students in the use of micropipetters and includes two methods of assessing student success. The second activity allows students to practice graphing and data analysis in the determination of protein concentration. Support materials for conducting these activities is available to any interested educators on the web site being created for the project: http://web.uri.edu/bi/.

Acknowledgements: Brown University's Project ARISE equipment loan program, Roberts Lab members, National Science Foundation Award IOS-1257047

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AGRICULTURAL BIOTECHNOLOGY AND GMOS: INFORMING THE DEBATE-A ONE CREDIT EDUCATIONAL ONLINE MODULE

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Biotechnology education has simply not kept pace with its science. The on-going anti-GMO debate is one example. A highly successful General Education OnLine course called *Issues in Biotechnology* has been developed to meet this need. This OnLine course has now been modularized and made further accessible as MOOCs intended for the general public, life sciences industry science and non-science staff, high school teachers and undergraduate students regardless of their major or degree program. There are no prerequisites. The Agricultural Biotechnology OnLine Module consists of nineteen video captured lectures with embedded PowerPoint slides followed by study guide questions. The series begins with twelve lectures providing a basic background for the biology and techniques necessary to inform the discussion about biotechnology generally as a field. This platform of educational materials has been created and allows for the presentation of independent modules on the various biotechnology topics. The Module on Agricultural Biotechnology consists of seven lecture lectures: I. Where Does Our Food Come From? II. DNA-based Biotechnology And Modern Agriculture, IIIa. Setting the Stage about Food and Agriculture, IIIb. Issues, Controversies and Concerns, IIIc. The Organic Food Debate, IV. The Ethics of Agriculture, V. Renewable Energy and the Future of Humanity. All entire course lectures and materials are free and can be viewed at www. lifeedu.us. The Course can be taken in three ways: (I) As a one (1) credit hour course for College Credit through the University of Rhode Island; (II) To receive a recognized Certificate through the University of Rhode or lifeedu.us; and, (III) As a Massive Open OnLine (MOOC) Course for FREE as an open educational experience.

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LABORATORY INTERNSHIPS IN PLANT BIOTECHNOLOGY: AN INQUIRY-DRIVEN EXPERIENTIAL LEARNING OPPORTUNITY IN AGRICULTURAL BIOTECHNOLOGY

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An undergraduate internship experience has been created as a model program to provide students real-world training and laboratory skills in plant biotechnology. The goal of this experience is to engage students in real world science in plant biotechnology in the context of an on-going research program providing undergraduate students the opportunity to learn current techniques applied in plant genetic engineering and agricultural biotechnology. The research program areas include: *Genetic Engineering of Corn for Nutritional Enhancement for African Germplasm* (HarvestPlus/CIAT) *Hybrid Technologies for Heterosis in Rice and Related Cereals* (NSF BREAD) and, *Genetic Improvement of Switchgrass, Biomass* (DOE). Students work in teams where "*The nature of science education needs to mirror the process of science itself*" in the context of this project-based experience, students learn significant laboratory skills and gain rigorous training. These laboratory skills include aspects such as laboratory safety, tissue and cell culture, aseptic technique, media preparation, bacterial cell preparation, DNA introduction methods, such as microprojectile bombardment and *Agrobacterium*-mediated transformation, analysis of transient and stable transformation comparisons, DNA isolation, reporter gene analysis, PCR and Southern blot analysis, photomicrography, data presentation and proper laboratory notebook record keeping. During the course, students contribute to Standard Operating Procedures (SOPs) for various techniques and protocols. This approach drives interest in underlying fundamentals through current and advanced technologies. Students learn science by actually doing it.

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A NOVEL γ-GLUTAMYL CYCLE IN PLANTS AND ITS ROLE IN PROVIDING TOLERANCE TO OXIDATIVE STRESS VIA GLUTATHIONE HOMEOSTASIS

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The ability of plants to tolerate environmental stresses has been associated with the role of glutathione. Glutathione (GSH) is an antioxidant crucial for biotic and abiotic stress management in all living cells. GSH prevents damages to essential cellular components caused by reactive oxygen species (ROS) such as free radicals and peroxides. Plants detoxify toxic metals through a GSH-dependent pathway. GSH homeostasis is maintained by the gamma-glutamyl cycle, which involves GSH synthesis, degradation and recycling of component amino acids. In *Arabidopsis thaliana*, the gene of interest, gamma-glutamyl cyclotransferase (GGCT), an enzyme which catalyzes the formation of pyroglutamic acid from dipeptides containing gamma-glutamyl is encoded by a ChaC-like protein with a cation transport regulator-like domain. There are three paralogs of this ChaC- like proteins in Arabidopsis (AtGGCT1, AtGGCT2;1 and AtGGCT2;2). Overexpressed lines of GGCT2;1 have been characterized to show high tolerance to cadmium and arsenite treatments due to enhanced GSH turnover and homeostasis during abiotic stress by recycling glutamic acid (*Paulose et al., 2013, Plant Cell 25: 4580–4595*). We have cloned and overexpress the remaining two, AtGGCT1 and AtGGCT2;2, genes from Arabidopsis. Further characterization of AtGGCT genes and their role in tolerance to other abiotic stresses is in progress. Similarly, we have identified and cloned homologous genes from *Camelina sativa*, which is an important oil seed crop and has tremendous potential for biofuel production. There are three homologs of GGCT2;1 in *Camelina sativa*. Our goal is to overexpress the ChaC genes in camelina to characterize their expression levels in response to oxidative stress caused as a result of abiotic stresses such as heavy metals, drought, high salt and ozone level. We have cloned and overexpress CsGGCT2;1 in camelina and transgenic plants will be evaluated for enhanced oxidative stress tolerance. Overexpression of GGCT genes in camelina will enable this crop to grow on marginal lands for biofuel production.

P33

DO MECHANOSENSITIVE CHANNELS FUNCTION IN ROOT-KNOT NEMATODE PARASITISM?

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Root-knot nematodes (RKN) are obligate, endoparasitic roundworms capable of infecting thousands of plant species, including many agriculturally important crops. Multiple phases of the RKN infection process cause mechanical stress on the root including; penetration, migration, giant cell formation and the swelling of the knots. These mechanical forces can be perceived in the plant by mechanosensitive channels. *Arabidopsis thaliana* contains 10 genes encoding MscS-Like (MSL) mechanosensitive channels, five of which (*MSL 4, 5, 6, 9,* and *10*) form the major plasma membrane mechanosensitive channel in the root. A mutant line lacking these five *MSL* genes has been created and is referred to as the quintuple *msl* mutant. The quintuple *msl* mutant supports less adult female nematodes than the Columbia wild type. Additionally, we observe defects in the early stages of nematode infection with fewer juvenile nematodes being retained in the root. Using acid fuchsin staining, the spatial distribution of the juvenile nematodes was examined and in the quintuple *msl* mutant nematodes they are predominately in the lower third of the root. Promoter:GUS fusion constructs for the five *MSL* genes revealed differences in expression during nematode infection. Complementation assays with MSL9 and MSL10 in the quintuple *msl* mutant background, also suggest functional differences between the MSL members. Currently, we are taking a closer look at MSL9. The *msl9* mutant shows changes in gene expression as tested by RT-PCR when infected with nematodes as compared to the Columbia wild type.

Acknowledgements: The project was supported by the Adrian Tinsley Program at Bridgewater State University to C.H. and A.B.

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GENE REGULATIONS AND ANTIOXIDANT ENZYME RESPONSES IN DEFENSE SYSTEM OF *ARABIDOPSIS THALIANA* TO NANOPARTICLE CERIUM AND INDIUM OXIDE EXPOSURE

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Rare earth elements (REEs) nanoparticles (NPs) have been widely applied in various products such as polishing agents, cosmetics and catalyst. The released NPs could inevitably cause a series of environmental problems as the demands of nanoproducts increased annually. However, the risk assessment of nanotoxicity in plants is still at the initial stage. In this study, the effects of cerium oxide (CeO_2) and indium oxide (In_2O_3) NPs exposure on *Arabidopsis thaliana* were investigated. After inoculation in 1/2 strength MS medium amended with 0-2000 ppm CeO_2 and In_2O_3 NPs for 25 days, both physiological and molecular responses were evaluated. Exposure at 250 ppm CeO_2 NPs significantly increased plant biomass but at 500-2000 ppm, plant growth was decreased by up to 85% in a dose dependent fashion. Malondialdehyde (MDA) production, a measure of lipid peroxidation, was increased by 2.5-fold at 1000 ppm CeO_2 NPs exposure. Interestingly, MDA production were unaffected by In_2O_3 NPs exposure. Expression of genes central to the stress response such as the sulfur assimilation and glutathione (GSH) metabolic pathway was determined by qPCR. In_2O_3 NPs exposure resulted in a 3.8-4.6 fold increase in glutathione synthase (GS) transcript production whereas CeO_2 NPs yielded only a 2-fold increase. It seems likely that the significantly greater metabolic response upon In_2O_3 NPs exposure was directly related to the decreased phytotoxicity relative to CeO_2 treatment. Meanwhile, altered antioxidant enzyme activities, which are able to scavenge reactive oxygen species (ROS) induced by both NPs, were measured at 250 and 1000 ppm treatments. Except superoxide dismutase (SOD), several antioxidant enzymes were highly induced to defend nanotoxicity. These antioxidant enzymes include catalase (CAT) and ascorbate peroxidase (APX), both of which mainly contribute to destructing ROS; phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD), involved to secondary metabolites in plants; glutathione S-transferase (GST), which was significantly induced at CeO_2 NPs exposure. These studies will be highly useful in understanding the fate, transport, and toxicity of manufactured NPs in the agricultural crops and to further develop strategies for mitigating the toxicity of these NPs in food crops.

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EXPLORING NEMATODE-INDUCED CHANGES IN TRANSPORTER GENE EXPRESSION

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Root-knot nematodes (*Meloidogyne spp.*) induce the formation of multinucleated cells, called giant cells, which serve as the exclusive source of nematode nutrition. Root-knot nematode-induced giant cells display special morphological characteristics including thickened and highly invaginated cell walls, dense cytoplasm, abundant ER, and numerous small vacuoles and mitochondria. These same characteristics are also shared with transfer cells that occur normally during plant development, such as in the seed. In order to understand the pathways involved in giant cell formation and function, we undertook a transcriptomic approach examining gene expression over a time course of nematode infection. RNA was isolated from giant cells and control root samples using laser capture microdissection. Agilent Arabidopsis microarrays were used to compare gene expression in the giant cells and control roots at 7, 14, and 21 days post-infection. After statistical analysis of the data, a list of giant cell up- and down-regulated genes was generated. After annotating the functional categories of the genes, we decided to concentrate on those involved in transport. The thickened cell walls and lack of plasmodesmata in giant cells requires that substances be moved by transport proteins into and out of the giant cell. T-DNA insertion Arabidopsis lines were used to examine the function of the identified transporter genes during nematode parasitism. Several of the T-DNA insertion lines have reduced nematode infestation levels, including those for ABCA9, ABCI8, CHX4, SLAC1, At1g04570, and At2g41190. A two-pronged approach, using both RT-PCR and promoter:GUS fusions, is being conducted to further characterize these genes during plant growth and development and nematode parasitism.

Acknowledgements: We thank R. Howard Berg at DDPSC for assistance with the microscopy and Lauren McIntyre and Alison Morse at the University of Florida for assistance with the bioinformatics. This project is funded by the National Science Foundation-Plant Genome Research Program #0821954 to C.G.T. and the Adrian Tinsley Program at Bridgewater State University to C.M. and C.D.

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NITROGEN FERTILIZATION CAUSES INCREASE IN DIVERSITY OF GENERA OF PHYLUM *ACIDOBACTERIA* AT HARVARD FOREST, MA

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In order to determine the effects of chronic nitrogen additions on plant and microbial communities of otherwise nitrogen-limited Northeastern forests a long-term nitrogen fertilization experiment was started in 1989 at Harvard Forest, Petersham MA (http://harvardforest.fas.harvard.edu/). Thirty soil samples (three treatments x two horizons x five subplots) were collected in 2009 from untreated (control), low nitrogen-amended (LN; 50 kg ha^{-1} yr^{-1}) and high nitrogen-amended (HN; 150 kg ha^{-1} yr^{-1})] plots. Using the technique of oligotyping, this study revealed the bacterial diversity that was undiscovered in our previous study using OTU clustering of 16S ribosomal RNA amplicons at 97% sequence homology (Turlapati et al. 2013). NMDS analysis on oligotypes revealed that the bacterial communities differed significantly among soil horizons and treatments. Whereas 11 oligotypes corresponding to Gp1were exclusively present in both soil horizons for LN this number was 25 for HN soils; similarly Gp2, only 9 oligotypes were exclusively present in both N-amended soils. For other subdivisions relatively small changes were observed. Previously undiscovered genera from these soil samples were identified; oligotypes corresponding to *Terriglobus* (Gp1) were discovered only in LN and HN soils; the ones corresponding to *Acidobacterium*, *Granulicella*, *Edaphobacter* (both Gp1) and *Bryobacter* (Gp3) were found in all three soils. The sequences belonging to *Edaphobacter* and *Terriglobus* of Gp1 were also significantly higher in HN-Org soils compared to control. No known bacterial genera were identified from Gp2, Gp4, Gp5, Gp6, Gp7, Gp10 and Gp13 subdivisions. In summary, significant differences were observed in the diversity of genera belonging to *Acidobacteria* within the two soil horizons as well as three treatment plots. Treatment effects were especially prominent in more abundant subdivisions (Gp1, Gp2, and Gp3).

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TRANSGENIC UPREGULATION OF POLYAMINE BIOSYNTHESIS IN WILLOW AND HYBRID *POPULUS* TO ENHANCE NITROGEN AND CARBON ASSIMILATION

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The primary objective of this project is to investigate whether upregulation of polyamine (PA) biosynthesis truly results in increased nitrogen (N) and carbon (C) assimilation, translates to increased biomass, and causes any significant physiological or metabolic consequences that will effect the tree's environment in a hybrid poplar (*P. nigra x maximowiczii* – clone NM6) and a willow (*Salix purpurea Var. Fish Creek)*. After successful initiation of willow into culture, upregulation in both species was accomplished via *Agrobacterium* mediated transformation with an inducible or constitutively expressed mouse ornithine decarboxylase (m*ODC*) gene. Successful transformation has been confirmed and trees will be transferred to the greenhouse for observation and testing during the growing season. Initial HPLC analyses will confirm whether the manipulated pathway actually causes increased PA production. We will then extend our current knowledge of the role of PAs by investigating links between PA synthesis and biomass accumulation, with anticipated applications to biofuel production, phytoremediation of toxins from contaminated soils, and riverbank stabilization. Increases in biomass as evidenced by weekly chlorophyll and soluble protein measurements and increases in C and N assimilation as demonstrated by CHNS analysis and radioactive $^{14}CO_2$ incorporation into leaves will signify further testing of wood properties, water usage, effects on soil microbial communities, and environmental risks like volunteerism and reproduction shifts, while unchanged biomass will direct us towards more qPCR and sequencing to pursue a genetic investigation of related pathways and possible regulatory mechanisms that could be preventing biomass accumulation.

Acknowledgements: I am grateful for help from Swathi Turlapati and support from the University of New Hampshire Graduate School Fellowship Programs.

P38

A SCREEN TO IDENTIFY SODIUM-INDUCED ROOT SKEWING MUTANTS IN *ARABIDOPSIS THALIANA*

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The roots of plants usually grow parallel to the gravity vector and the enzyme Protein Phosphatase 2A (PP2A) is required for normal root growth. PP2A consists of three subunits: a scaffolding A subunit, a regulatory B subunit and a catalytic C subunit. Previous research showed that mutations in particular A or C subunits caused a novel sodium-dependent root growth phenotype, known as skewing. When roots skew, they grow at an angle to the gravity vector. Our objective is to learn more about this important root growth pathway by identifying additional genes whose disruption leads to sodium-induced root skewing. We are using a multi-part mutant screen to accomplish this objective. In the primary screen, plants with a root-skewing phenotype on sodium-supplemented medium are initially identified. The secondary screen verifies that the potential mutants from the primary screen have a reproducible phenotype. The primary screen has been completed and 2,200 putative mutants were selected from about 143,750 seedlings. Of the 2,200 mutants, to date, 800 have been re-tested in the secondary screen where 37 have shown a reproducible root skewing phenotype. In the tertiary screen, mutants that pass the secondary screen will be evaluated to determine whether the root skewing is sodium specific.

P39

MOLECULAR GENETIC AND BIOCHEMICAL ANALYSIS OF THE STRESS RESPONSE ACTIVITY OF CYP72A ENZYMES FROM ARABIDOPSIS

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Plant metabolic responses to environmental conditions require thousands of enzymes that must work in delicately balanced concert to facilitate plant growth and defense against pests. Plants with the most tightly regulated metabolism and growth are more likely to thrive. The cytochrome P450s (CYPs) are a group of enzymes that catalyze biochemical reactions in all organisms, and they are particularly important in plant secondary metabolism. There are hundreds of CYPs in plants, and they are grouped into subfamilies based on genetic similarity. Our approach is to combine a molecular genetic analysis with biochemistry to describe subtle differences in apparently redundant CYPs from Arabidopsis. The CYP72A subfamily appears to contribute to producing defensive secondary metabolites in response to stress and herbivory. The subfamily is found in all plants but appears to be diversifying in recent evolutionary history. We are examining single, double and triple mutants to determine the role the enzymes play in response to abiotic and biotic stresses in Arabidopsis. We are examining the structural constraints of the subfamily that will provide insight into the biochemical activity of the group. We are also optimizing expression of the CYP72A enzymes in yeast for direct analysis of substrate interactions. Our studies in Arabidopsis provide a framework for understanding the function of these enzymes in secondary metabolism in other plants.

Acknowledgements: This project was funded by the TCNJ MUSE program and Research Corporation for Science Advancement.

CONTRIBUTED TALKS

T1

CHANGING B-CLASS MADS BOX PROTEIN-PROTEIN INTERACTIONS ACROSS EVOLUTIONARY TIME: WHEN, HOW, AND WHY?

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Altered protein-protein interactions are often invoked as a prime mechanism for effecting evolutionary change, but empirical examples remain scarce. We have uncovered evolutionary lability in a regulatory protein-protein interaction, between APETALA3-like ($AP3^L$) and PISTILLATA-like (PI^L) proteins, in the monocot order that contains the grasses, the Poales. The $AP3^L$-PI^L interaction represents a key node in the floral developmental network: $AP3^L$ and PI^L proteins are B-class MADS box proteins necessary for both stamen and second whorl organ development in a number of species. $AP3^L$ and PI^L proteins act as obligate heterodimers in many plants, spanning much of flowering plant diversity. Intriguingly, there is evidence that PI^L homodimerization is the ancestral state, and that obligate $AP3^L$-PI^L heterodimerization evolved more than once in the Poales. We pinpointed when in the evolutionary history of the Poales obligate $AP3^L$-PI^L heterodimerization arose. Through domain swap and site-directed mutagenesis experiments, we have identified the protein domains and amino acid residues responsible for these shifting interactions. We will discuss these results in the context of the reigning paradigm for understanding molecular evolution.

T2

COMPUTATIONAL AND EXPERIMENTAL FRAP ANALYSES OF MYOSIN XI DEPENDENT VESICULAR TRANSPORT SHOWS COUPLING WITH F-ACTIN IN POLARIZED CELL GROWTH

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Plant polarized cell growth is driven by the trafficking of secretory vesicles to the site of cellular expansion. Although the actin cytoskeleton and its associated motor myosin XI are heavily implicated in this process, little is known about their relative dynamics and binding properties in vivo. Here we applied fluorescent recovery after photo-bleaching (FRAP) techniques to the tip-growing cells of the moss Physcomitrella to investigate myosin XI dynamics and its relation to secretory vesicles. To complement our FRAP experiments, a three-dimensional Brownian dynamics simulation of FRAP was used to gain further insight into the molecular transport of myosin XI and vesicles. Our results indicate that the dynamics of myosin XI at the cell apex differ from those measured at the sub-apical region. Specifically, the rate of fluorescent recovery was more rapid at the sub-apical region, indicating that a fraction of myosin XI is less mobile at the tip. To evaluate if the reduction on motility is dependent on F-actin, we depolymerized the actin using latrunculin B. This treatment increased the mobile fraction of myosin XI at the cell apex, but not to the levels of the sub-apical region. Our simulations indicate that this discrepancy can be explained by cell boundary effects. Simulations have allowed us to estimate diffusion coefficients for myosin XI and vesicles, and the fraction of myosin XI molecules associated with vesicles. Our experimental results and simulation data support a model where myosin XI, secretory vesicles, and F-actin act cooperatively to drive polarized secretion and growth.

Acknowledgements: This research was funded by NSF.

T3
NITROGEN ASSIMILATION IN SHRUB WILLOW FERTILIZED WITH DIFFERENT FORMS OF NITROGEN VIA FOLIAR AND SOIL APPLICATION
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We examined foliar application of fertilizer using different nitrogen sources in shrub willow. Shrub willows are known for their high biomass production in short time periods, ease of vegetative propagation and coppicing ability after multiple harvests. Our goal was to determine 1) if foliar application is an effective form of fertilization and 2) if different nitrogen sources are assimilated differently by the plant. Most crops are fertilized through the soil however less than 50% of the applied fertilizer actually makes it into the plant it was intended for; the remaining nitrogen is lost to the environment in groundwater, surface water and the atmosphere via microbial activity and volatilization. Foliar fertilization reduces the risk of nitrogen loss to the environment through leaching and microbial interaction. We examined the plants ability to utilize five different forms of nitrogen (urea, Nitamin, GPG, ammonium nitrate and arginine) by quantifying foliar polyamines, total protein, total N and carbon and biomass. We found rapid short-lived increases in polyamines (mainly putrescine) within three days of treatment in all foliar treatments as well as long term (2 to 5 weeks) increases in total protein and total nitrogen in most treatments. None of the foliar treatments produced significantly more biomass than the unfertilized control however the foliar urea treatment did result in a 13% increase in total biomass. We conclude that foliar applied nitrogen has the potential to be the main source of nitrogen for the plant and that urea is the most effective nitrogen form.

T4
COMPREHENSIVE ANALYSIS OF CIRCADIAN CLOCK REGULATED PROTEIN DEGRADATION USING A DECOY F-BOX STRATEGY
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The circadian clock is a critical endogenous timing mechanism that integrates environmental information to assign biological processes to specific times of day. All clocks have a conserved architecture that includes inputs that provide time-of-day information, the clock mechanism itself, and outputs that are biological processes parsed to specific times. In plants most studies have focused on the transcriptional feedback loops that make up the core clock and connect to outputs by controlling gene expression. Little is understood about the mechanisms that control rhythmic protein degradation that is critical to oscillating protein abundance of clock components and factors involved in output processes. Our lab is utilizing a "decoy" strategy to understand the genetic role of F-box proteins in the rhythmic degradation of clock proteins and to define F-box interactomes to identify the full suite of F-box substrate proteins and interacting partners. Initial genetic and mass spectrometric results will demonstrate the robustness of the decoy technique in defining function and identifying previously elusive substrates and interaction partners. Determining how protein stability is rhythmically controlled will move the circadian field vertically by expanding our understanding of how the clock integrates and responds to environmental information at the post-translational level.

T5

SMALL-MOLECULE DISSECTION OF VEIN PATTERNING IN ARABIDOPSIS

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Genetic screens have revealed some of the factors affecting leaf vein patterns; however, the critical role of veins in transporting water and essential nutrients suggests that some key regulators may be genetically redundant and thus undetected in forward genetic screens. As an alternative means to identify novel genetic regulators of vascular patterning, we screened over 5,000 structurally diverse small molecules from LATCA and other chemical libraries for their ability to alter Arabidopsis foliar vein patterns. Many compound-induced phenotypes were observed including vein networks with an open reticulum, decreased or increased vein number and thickness, ectopic xylem cells, and misaligned, misshapen or non-polar vascular cells. Vascular patterning is established by the flow of auxin, which relies on the membrane-localized PIN auxin efflux carriers. The observed vein pattern defects from several identified compounds, apparently novel auxin transport inhibitors (ATIs), appear to be due to the disruption of PIN1 and PIN2 trafficking, based on an examination of BFA-induced PIN endosomal accumulation. Small-molecule profiling using physiological assays with reporter systems and mutants demonstrates that novel ATIs affect distinct targets, which is consistent with their diverse structures. In contrast, hypervascularizing compounds form a cluster as shown by chemical dendrogram analysis. Furthermore, Q-RT PCR analyses show that several compounds have direct transcriptional influence on specific hormonally regulated genes. Structural similarity searches using chemical databases for target identification reveal a role for serine hydrolases in vein patterning. This study demonstrates the utility of a phenotypically-based small molecule screen to perturb a genetically controlled developmental process.

Acknowledgements: This research was funded by NSF.

T6

A NEW APPROACH TO MEASURE HYDRAULIC RESISTANCE IN PLANTS

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Measuring xylem hydraulic resistance in plants is a common procedure that relies on well-established methods. Results from comparative measures of xylem hydraulic conductivity using traditional and a new protocol on eight woody species will be discussed. The new method was developed to reduce errors associated with samples that are: shorter than their xylem conduit lengths; and from fluid moving through artificially created flow paths when measurements are made on samples that are excised on both ends, such as fluid moving through xylem conduits located in older vascular growth rings that may not be conducting in the intact samples. The new method produced much lower values of hydraulic conductivity in 7 of the 8 species studied. However, there was a large species-dependent range observed between the two methods. For example, the new method produced conductivity values that were two times lower for *Acer rubrum* samples compared to traditional hydraulic methods. And in some species, the differences between the two methods were only about 10%. For *Robinia pseudoacacia* conductivity values were found to be the same for both methods. Reasons for these variations will be discussed.
Acknowledgements: Samuel Hodgson and Michael Guidi

T7

IDENTIFICATION AND CHARACTERIZATION OF DIACYLGLYCEROL ACYLTRANSFERASE TYPE-1 (DGAT1) FROM *CHLORELLA VULGA*RIS

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Current research on oil biosynthesis in microalgae is focused on a small number of species, including *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species. The strain Chlorella vulgaris exhibits a desirable biodiesel profile containing mainly saturated fatty acids[1], which highlights the highly oxidative stability of its oil. Also it has been identified as a high oil producing species by the Aquatic Species Program. These properties make *C. vulgaris* a promising candidate for biodiesel production.

In this study, we investigated a potential bottleneck to triacyl glycerol (TAG) biosynthesis - diacylglycerol acyltransferase type-1 (DGAT1) - which catalysis the last step in TAG production. The CvuDGAT protein level was previously shown to be highly increased in algae grown under nitrogen deprivation[2]. In addition to identification and characterization of the C. vulgaris DGAT1 (CvuDGAT1) gene, its impact in TAG production was investigated by transforming a quadruple disrupted non-oil-producing yeast mutant strain H1246 with our CvuDGAT1 gene.

After obtaining the CvuDGAT1 sequence, its protein sequence was characterized using various bioinformatic programs. The yeast mutant H1246 was transformed with the yeast plasmid pYES2 containing our CvuDGAT1 gene sequence.

We found that the CvuDGAT1 protein (460 aa) shares many characteristics of other DGAT1 sequences in microalgae and plants. These include nine predicted trans-membrane domains, the conserved predicted acyl-CoA binding site motif[3], and the highly conserved histidine, which is predicted to be part of the active site in DGAT1[4]. In addition, the expression of CvuDGAT1 gene restored oil production in the non-oil-producing yeast mutant H1246. In summary, we successfully sequenced and demonstrated the enzymatic function of CvuDGAT1 for TAG biosynthesis.

Acknowledgments: This work was supported in part by the Catalyst Grant from the Massachusetts Clean Energy Center to C. Lee-Parsons and in part by a fellowship from the Leibniz Graduate Academy to L. Kirchner.

1. Mallick, N. et al. (2012) J. Chem. Technol. Biotechnol 87:137-145
2. Guarnieri, M. T. et al. (2011) PLoS ONE 6:E25851
3. Turchetto-Zolet, A. C. et al. (2011) BMC Evolutionary Biology 11 (http://www.biomedcentral.com/1471-2148/11/263, status:02/13/2014)
4. Hoffmann, K. (2000) Trends Biochem Sci 25:111-112

T8

THE CHLOROPLAST AS A PLATFORM FOR METABOLIC ENGINEERING.

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Most of the proteins in the chloroplast are encoded in the nucleus and transported to the organelle. However, a few key functions are encoded by the plastid's genome: an independent expression system, the large subunit of the carbon-fixing enzyme RuBisCO and key components of the photosystems. These genes are organized in a hybrid bacterial/eukaryotic fashion: Genes are expressed in operons, with bacterial-like promoters, but also contain introns and require transcription terminators. These features make the chloroplast an attractive host for metabolic engineering, both in terms of pathways that make the plant grow better, but also for the production of energy-intensive products like biofuels. Two particularly interesting bacterial systems are the nitrogen fixation cluster from *Klebsiella oxytoca* and the 3-hydroxypropionate carbon fixation bi-cycle from *Chloroflexus aurantiacus*. But before any synthetic biology can be performed in the chloroplast, the toolbox for heterologous gene expression must be improved. Transformation methods, promoters, terminators, ribosome binding sites are not well characterized, both for expression in the chloroplast genome as well as chloroplast-targeted nuclear genes. We have successfully transformed positive controls of chloroplast-targeted nuclear GFP, both transiently via gene-gun and stably through *Agrobacterium* transfection. The expression of larger pathways hinges on identification and characterization of more parts, which come from the higher-plant *Arabidopsis* and *Nicotiana* genomes, and orthogonal sequences from the chloroplast and cyanobacterial *Synechococcus* genomes. As we have a limited toolbox of existing promoters, RBS's, terminators, and chloroplast transit tags, we have designed our first constructs taking advantage of what is already known and will refine it more as we discover more useful chloroplast expression parts.

Acknowledgements: Synthetic Biology Engineering Research Center (National Science Foundation); Advanced Research Projects Agency – Energy (Department of Energy)

T9

PHYLOGENETIC AND FUNCTIONAL ANALYSIS OF THE CYP72A ENZYMES CONTRIBUTING TO SECONDARY METABOLISM IN FLOWERING PLANTS

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Plant secondary metabolites have provided the arsenal necessary for adaptation to life on land and survival from competition and attack. Cytochrome P450 monooxygenases (CYPs) are the enzymes have been implicated in many aspects of secondary metabolism, but much is still unknown about the biochemical capabilities of this large class of enzymes. Plant genome sequencing has revealed the presence of thousands of CYP genes with an average of about 300 genes per plant. Whole genome comparisons and microarray data allow CYPs to be organized based on gene structure, protein sequence, and similarities in expression patterns. These data provide insight into the CYP diversity in plants, but little is known about the connections between sequence similarity and biochemical function within large groups of closely related CYPs. The CYP72A subfamily appears to have members in all angiosperms and provides the potential for a variety of biochemical functions in each plant. The purpose of this study was to examine evolutionary relationships within the CYP72A subfamily as the framework for predicting functional diversity in the substrate binding sites of each enzyme. Our phylogenetic analysis shows relationships between CYP72A sequences from 34 angiosperm species. Our data supports the hypothesis that all CYP72As are derived from a common ancestor prior to the monocot/eudicot split. We used the phylogenetic tree to predict biochemical potential within the CYP72A subfamily and to make predictions for functions specific to smaller clades of CYP72A enzymes. This study provides insight into the evolution of secondary metabolism controlled by CYPs in angiosperms.

Acknowledgments: This project was funded by the TCNJ MUSE program and Research Corporation for Science Advancement.

T10

AN OPTIMIZED TRANSFORMATION METHOD FOR ESTROGEN-INDUCIBLE GFP EXPRESSION IN *CATHARANTHUS ROSEUS* HAIRY ROOTS

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The medicinal plant, *Catharanthus roseus*, is the source of several pharmaceutically valuable alkaloids, including two powerful anticancer alkaloids, vincristine (VCR) and vinblastine (VBL). These alkaloids are produced only in *C. roseus* and at extremely low levels (0.0002 wt%). Due to low levels, the isolation of these compounds is both laborious and costly, ranging from $4 to $60 million/kg. Despite these barriers, these alkaloids have been effectively used to treat cancer for over 50 years.

To improve alkaloid production in *C. roseus*, the expression of several enzymes and transcription factors involved in alkaloid biosynthesis have been genetically engineered using a constitutive expression system (cauliflower mosaic virus 35S promoter). An inducible system, however, provides many benefits over a constitutive system. In an inducible system, the timing of transgene expression can be controlled, clonal variation in negative control lines is avoided, and deleterious effects such as growth retardation can be circumvented.

A glucocorticoid-inducible system has been established and successfully used to express transgenes in *C. roseus* hairy roots. However, growth defects in Arabidopsis and rice have been attributed to one or more components of the glucocorticoid-inducible system. Therefore the estrogen-inducible (XVE) system is an alternative inducible system.

The process of obtaining stable transgenic hairy roots through *Agrobacterium*-mediated transformations can be timely and inefficient. Here, we report an optimized method of establishing transgenic *C. roseus* hairy roots (33% efficiency) under the estrogen-inducible XVE system driving GFP expression (Fig. 1).

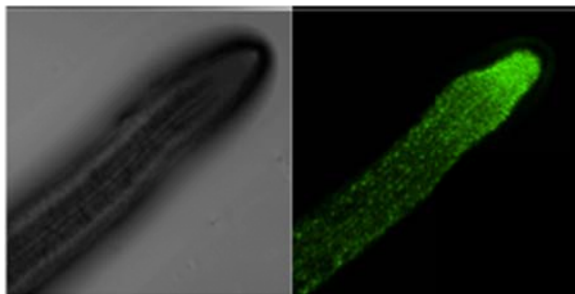

Figure 1: Transgenic hairy roots expression GFP after induction

Acknowledgements: This work was supported by the National Science Foundation (NSF CBET Award #1033889). The authors thank Dr. Nam-Hai Chua (The Rockefeller University, New York) for providing the pER8-GFP vector.

T11

DEVELOPING A WHOLE PLANT *ARTEMISIA ANNUA* ANTIMALARIAL THERAPEUTIC.

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The GRAS medicinal plant *Artemisia annua* L. produces the sesquiterpene lactone, artemisinin. The current therapy for malaria is artemisinin + an older drug: artemisinin combination therapy (ACT). In *Plasmodium chabaudi*-infected mice, dried leaves of *A. annua* are more potent than equal amounts of pure artemisinin and may also prevent artemisinin drug resistance from emerging. We call this whole plant therapy pACT: plant-based artemisinin combination therapy. Pharmacokinetics of artemisinin in healthy and infected mice given either pure artemisinin or pACT is very different and also showed that > 40 fold more artemisinin enters the blood when plant material is present. The presence of the plant matrix enhanced the bioavailability of artemisinin in the serum. Dried leaves in capsules or formed as tablets given to African malaria patients were also efficacious. Flavonoids, phenolic acids, monoterpenes and other artemisinic metabolites found in the plant have mild antimalarial activity. Some are reported to synergize with artemisinin to enhance the drug's efficacy. In simulated digestion studies the effects of cellulose and gelatin capsules, sucrose, 4 oils, and 3 staple grains (rice, corn, and millet) were studied to determine their effect on AN and flavonoid release into the liquid phase of the intestinal stage of digestion. Compared to pACT alone, both sucrose and oil enhanced release of flavonoids by 100%, but did not affect release of artemisinin. In contrast both capsule types, and corn and millet meal, significantly reduced the amount of artemisinin, but had no effect on flavonoids. Based on Massachusetts field trials, it is estimated that > 500,000 patients could be treated from plants grown on 1 ac of land. Taken together these results show how a simple herbal remedy could be used as a controlled, inexpensive, sustainable and efficacious orally delivered therapeutic for treating malaria and other artemisinin susceptible diseases.

Acknowledgements: Worcester Polytechnic Institute and University of Massachusetts Center for Clinical and Translational Science (CCTS) partially funded this project. This project was also partially supported by Award Number NIH-2R15GM069562-03 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

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