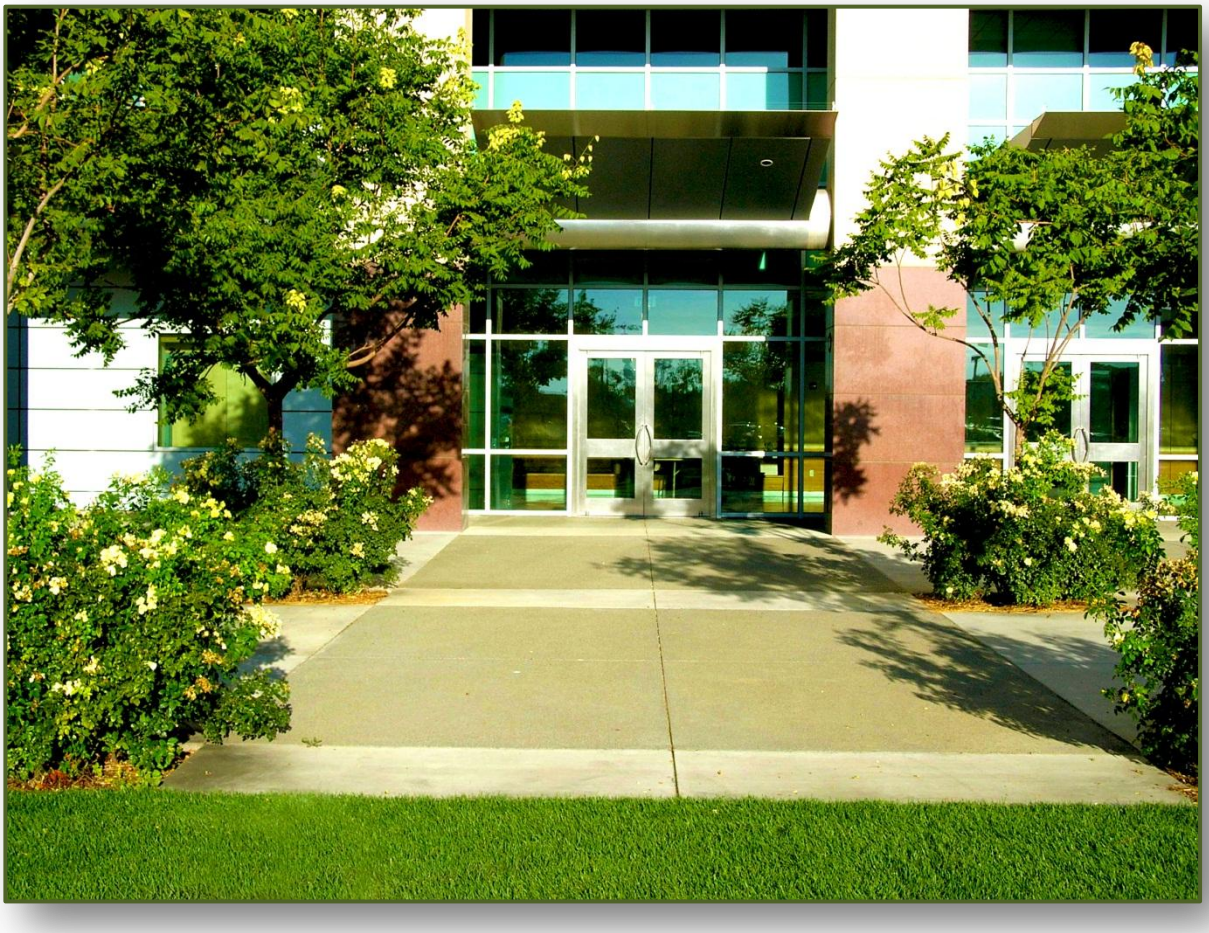
CREATE

IGERT

COLLABORATIVE RESEARCH & EDUCATION IN AGRICULTURAL TECHNOLOGIES & ENGINEERING

2010-2011
CREATE-IGERT SYMPOSIUM



January 11 - 12, 2011

UC Davis Genome Center

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2011 CREATE-IGERT SYMPOSIUM PARTICIPANTS

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**CREATE-IGERT Faculty Trainer*
***CREATE-IGERT Trainee*

Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) IGERT Symposium and Distinguished Lecture

January 11 – 12th, 2011 Genome Center, UC Davis

Welcome to the 2010-2011 CREATE-IGERT Distinguished Lecture and Symposium!

The Integrative Graduate Education and Research Traineeship (IGERT) program is a National Science Foundation program that encourages new approaches to interdisciplinary graduate education to prepare students to tackle complex, multifaceted real-world problems. The Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) IGERT, is a multi-institutional, international educational partnership between UC Davis, Tuskegee University, the National University of Ireland, Galway, the National University of Ireland at Maynooth, and the Teagasc Oak Park Research Centre, in Carlow, Ireland. CREATE integrates training in the plant sciences, molecular biology and engineering, to advance research and catalyze breakthroughs in the sustainable use of plants for production of non-food products ranging from biofuels to vaccines. In addition to the underlying scientific and engineering principles, trainees develop an understanding of the complex interconnected issues (environmental, ecological, sustainability, public/societal concerns, global impact, regulatory, intellectual property), preparing them as the research, educational, business, and policy leaders of the future.

Thank you for joining us as we honor our 2010-11 trainees and CREATE-IGERT affiliates (formerly funded trainees and other students working in faculty trainer labs), as well as our Tuskegee partners, faculty trainers, industry affiliates, and this year's Distinguished Lecturer, Dr. Roger Beachy, Director of the National Institute of Food & Agriculture.

I'd especially like to thank Dr. Denneal Jamison-McClung, CREATE-IGERT Program Coordinator and Associate Director of the Biotechnology Program, and Dr. Judith Kjelstrom, Director of the Biotechnology Program, as well as the Biotechnology Program Staff, Marianne Hunter and Demian Sainz, for all of their hard work in organizing this symposium.

The CREATE program is made possible through funding by the National Science Foundation (DGE-0653984), and support from the UC Davis Office of Research, Office of Graduate Studies, Biotechnology Program and Department of Chemical Engineering & Materials Science.

With warmest regards,

Karen McDonald
Director, CREATE IGERT Program
Professor, Chemical Engineering & Materials Science

CREATE-IGERT Distinguished Lecture and Symposium Schedule
January 11-12th, 2011
Genome Center, UC Davis

8:30-9:00am Registration & Coffee

9:00-9:10am Welcome

9:10-9:25am Overview of the CREATE-IGERT Training Program (Prof. Karen McDonald, CREATE-IGERT Director)

9:25-9:35am CREATE-IGERT Research Experiences for Undergraduates (Prof. Jean VanderGheynst)

Oral Presentations

9:35-10:00am Lucas Arzola, McDonald Laboratory, UC Davis
"Transient Co-Expression of Post-Transcriptional Gene Silencing Suppressors Increased In Planta Expression of a Recombinant Anthrax Receptor Fusion Protein"

10:00-10:25pm Dominique Gales, Yates Laboratory, Tuskegee University
"Investigation in the Role of Sweet Potato Leaf Extract"

10:25-10:40am Coffee Break

10:40-11:05am LaKisha Odom, Ankumah Laboratory, Tuskegee University
"Evaluation of Non-Agrobacterium Tumefaciens-Mediated Transformation in Three Plant Models: Tobacco, Arabidopis, and Wheat"

11:05-11:30am Steven Samuels, Egnin Laboratory, Tuskegee University
"Development of Transgenic Sweet Potato [Ipomoea Batatas (L Lam)] Expressing Synthetic Lytic Peptide Genes Jc41n and Jc41nd as a Plant-Based Treatment Regimen Against HIV Replication"

11:30-1:15pm Lunch – Poster Viewing

1:15-1:40pm Geoffrey Benn, Dehesh Laboratory, UC Davis
"*Identifying the Biochemical and Molecular Components of Plant Stress Perception"*

1:40-2:05pm Marta Bjornson, Dehesh Lab/Dandekahar Lab, UC Davis
"Production of Chimeric AntiMicrobial Proteins In Planta"

2:05-2:30pm	J. Mitch Elmore, Coaker Laboratory, UC Davis *"Quantitative Proteomics Reveals Dynamic Changes at the Plasma Membrane During Plant Immune Responses"*
2:30-2:55pm	Rachel Kerwin, Kliebenstein Laboratory, UC Davis *"Investigating the Importance of Natural Variation in the Glucosinolate Pathway using Arabidopsis thaliana"*
2:55-3:00pm	Closing Remarks – Prof. Karen McDonald
3:00-4:00pm	Tour of CREATE-IGERT Lab (Larry Joh) – Walk to Mondavi Center, Vanderhoef Studio Theater
4:00-6:00pm	Chancellor's Colloquium Distinguished Speakers Series *Dr. Roger Beachy, Director of NIFA "Support for Agricultural Research in the Beltway"*

IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE)

(NSF Award DGE0653984)

UC Davis P.I.s & Co P.I.s

Karen McDonald, Principal Investigator – UC Davis
Abhaya Dandekar, Co-Principal Investigator – UC Davis
Martina Newell-McGloughlin, Co-Principal Investigator – UC Davis
Pamela Ronald, Co-Principal Investigator – UC Davis
Jean VanderGheynst, Co-Principal Investigator – UC Davis
Denneal Jamison-McClung, Program Coordinator – UC Davis
Larry Joh, Program Engineer – UC Davis

Tuskegee University P.I.s & Co P.I.s

Luther Williams, Principal Investigator – Tuskegee University
Jesse Jaynes, Co-Principal Investigator – Tuskegee University
C.S. Prakash, Co-Principal Investigator – Tuskegee University
Deloris Alexander, Program Coordinator – Tuskegee University

IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE)

NSF Award DGE-0653984
August 15, 2007 – July 31, 2012

UC Davis has been awarded the multi-institutional IGERT: Collaborative Research and Education in Agricultural Technologies and Engineering (CREATE) grant from The National Science Foundation in the amount of $599, 824. The grant is under the direction of Karen A. McDonald; Department of Chemical Engineering, with co-PIs: Abhaya M. Dandekar, Department of Plant Sciences; Jean S. VanderGheynst, Department of Biological and Agricultural Engineering; Martina Newell-McGloughlin, UC BREP; and Pamela C. Ronald, Department of Plant Pathology. The lead institution is the University of California at Davis, Davis, CA and collaborating institutions are Tuskegee University, Tuskegee, AL (Luther S. Williams, PI); National University of Ireland, Maynooth, Ireland (Dr. Phil Dix, PI); Teagasc Oak Park Research Centre, Carlow, Ireland (Dr. James Burke, PI).

The IGERT program, entitled Collaborative Research and Education in Agricultural Technologies and Engineering (**CREATE**), will provide a structured and well-integrated graduate research and educational training program focused on a **unifying theme** of **transgenic plants and *in-vitro* plant systems for the production of industrial non-food products and biopharmaceuticals. Research focus areas are 1) Plant-Made Products, 2) Biofuels and Biorefineries, and 3) Environmental Sustainability.** Across the three broad focus areas**,** specific attention will be paid to **the scientific, engineering, environmental, regulatory, economic, intellectual property, societal and global issues** associated with plant biotechnology**.**

The **Project Objectives** for CREATE-IGERT are to:

1. CREATE a **framework for interdisciplinary graduate training** that will foster an environment for revolutionary breakthroughs at the interface of plant science, biotechnology, and engineering.
2. CREATE **new scientific knowledge**, engineering technologies, tools, methods, processes, and global understanding to advance the fields of plant science, biotechnology, engineering and areas at the interface of these disciplines, particularly those related to the underlying theme.
3. CREATE and cultivate the **integrative skill set** in graduate student trainees, faculty trainers, and postdoctoral scholar participants using the underlying theme as the focus.
4. CREATE a training program to **attract, retain, and graduate doctoral students from diverse backgrounds** who are not only top-rated scientists and engineers but also have the variety of skills and understanding to approach problems from integrated perspectives, allowing them to become the academic, industrial, national laboratory, and/or policy leaders in areas related to the unifying theme.
5. CREATE a **Masters to PhD Bridge Program** that strengthens research and graduate training linkages between UC Davis and Tuskegee University in areas related to plant biotechnology and provides a guided transition for MS students at Tuskegee into doctoral programs at UC Davis.

To apply for IGERT support, students **must be a member of the DEB**

Designated Emphasis in Biotechnology Program (DEB)

Goals and Mission of the DEB

The Designated Emphasis in Biotechnology (DEB) is an inter-graduate group program that allows Ph.D. students to receive and be credited for training in the area of biotechnology. The DEB provides a nurturing interactive environment to promote integration of multiple disciplinary approaches to the conduct of research and to promote learning in biotechnology. The mission is to prepare well-educated students to approach problems with creativity and flexibility. The program will provide tools for the students to be leaders, visionaries, entrepreneurs, researchers and teachers in the broad area of biomolecular technology.

DEB Mission:

- To provide well-coordinated, cross-disciplinary training of graduate students in critical areas of biomolecular technology research.
- To promote interdisciplinary research environments that integrate basic biological science, engineering and computational disciplines.
- To allow cross-disciplinary training and trainee experience in a biotechnology company or cross-college laboratory.

Students come from a wide array of disciplines: Participating graduate programs currently include **27 programs**: Agricultural and Environmental Chemistry; Animal Biology; Applied Science; Biochemistry, Molecular, Cellular & Developmental Biology; Biological Systems Engineering; Biomedical Engineering; Biophysics; Chemical Engineering; Chemistry; Civil and Environmental Engineering; Comparative Pathology; Electrical & Computer Engineering, Entomology; Food Science Technology; Genetics; Immunology; Materials Science & Engineering; Mechanical and Aeronautical Engineering; Microbiology; Molecular, Cellular & Integrative Physiology; Neurosciences; Nutritional Biology; Pharmacology & Toxicology; Plant Biology; Plant Pathology; Soils & Biogeochemistry and Statistics. The DEB program supplements a student's Ph.D. curriculum and those completing the program will obtain an official designation on their diploma & transcript indicating a qualification in biotechnology. Example: **Doctoral Degree in Microbiology with a Designated Emphasis in Biotechnology**

***CREATE-IGERT Trainees must be enrolled in the DEB**

Oral Presentations

TRANSIENT CO-EXPRESSION OF POST-TRANSCRIPTIONAL GENE SILENCING SUPPRESSORS INCREASED *IN PLANTA* EXPRESSION OF A RECOMBINANT ANTHRAX RECEPTOR FUSION PROTEIN

Trainee: Lucas Arzola
Faculty Trainer: Karen McDonald
Campus: UC Davis

Lucas Arzola[1*], Junxing Chen[1], Kittipong Rattanaporn[1], James Maclean[2], and Karen McDonald[1]
[1] Department of Chemical Engineering and Materials Science, University of California, Davis
[2] Planet Biotechnology, Inc., 25571 Clawiter Road, Hayward, CA 94545

Potential epidemics of infectious diseases and the constant threat of bioterrorism demand rapid, scalable, and cost-efficient production of therapeutic proteins. Molecular farming of tobacco provides a cost-effective alternative for production of pharmaceutical proteins. *Agrobacterium tumefaciens* mediated transformation, utilizing agroinfiltration, can be used to rapidly induce the transient expression of a protein of interest. We have developed a transient production platform for a novel anthrax receptor decoy protein (immunoadhesin), PBI-220, in *Nicotiana benthamiana* plants. This chimeric fusion protein is composed of the von Willebrand factor A (VWA) domain of human capillary morphogenesis 2 (CMG2), an effective anthrax toxin receptor, and the constant region of human immunoglobulin G (IgG). PBI-220 mitigates the effects of anthrax by preventing production of the deadly anthrax toxins.

We have evaluated, in intact plants and detached leaves, the co-expression of PBI-220 with nine different viral suppressors of post-transcriptional gene silencing (PTGS): p1, p10, p19, p21, p24, p25, p38, 2b, and HCPro. *Nicotiana benthamiana* plants and leaves were sampled at 3.5 days, 7 days, and 14 days post agroinfiltration. ELISA and Bradford assay were performed to quantitatively measure the PBI-220 protein concentration and total soluble protein concentration, while Western Blot was used to confirm the identity of the expressed PBI-220. Overall, transient expression of PBI-220 was higher on intact plants than detached leaves. It was observed to be highest with p1 co-expression at 3.5 days, resulting in a maximum production of 0.56 g PBI-220 per kg fresh weight of leaf. Co-expression with certain PTGS suppressors - particularly p1, p19, and p21 - significantly increased PBI-220 expression levels.

INVESTIGATION IN THE ROLE OF SWEET POTATO LEAF EXTRACT

Trainee: Dominique N. Gales
Faculty Trainer: Dr. Clayton Yates
Campus: Tuskegee University, Tuskegee, AL

Dominique N. Gales[1*], Leah O'Neal[1], Ritu Aneja[2], Dr. Clayton Yates[1], and Dr. Timothy Turner[1]
Department of Biology and Carver Research Foundation[1], Tuskegee University, Tuskegee, AL

Prostate cancer is the most common malignancy in American men and the second leading cause of deaths from cancer. According to recent reports it has been indicated that there have been are 217,730 new cases and 32,050 deaths in 2010. Metastasis, the uncontrollable spread of cells that invade other parts of the body, particularly the bones and lymph nodes makes it difficult to successfully treat patients in an effective manner after its occurrence. It is therefore vital to have early markers for prostate cancer. Our possible contributor to lower the rates of prostate cancer in African American men is higher consumption of a typical vegetable in our population. Ongoing research concerning Beta-Carotene fibers, Vitamins A, C and E, Iron, Calcium, Proteins and Zinc has identified the Sweet Potato (*Ipomoeas Batatas*) as becoming a potential anticancer component of *convolvulaceae* vegetables. Sweet Potatoes have been shown to reduce many disease related to inflammation, asthma, arthritis and chronic diseases such as, cardiovascular disease and cancer. Previous data in our lab has shown that treatment of prostate cancer cells with sweet potato leaf extract effectively decreases cell proliferation. Other collaborators have also shown that sweet potato green extract (SPGE) exerts significant growth-inhibitory effects in a repertoire of prostate cancer cell lines. Detailed studies to dissect molecular mechanisms demonstrated that SPGE impeded cell-cycle progression, impaired reproductive capacity, modulated cell cycle and apoptosis regulatory molecules, and induced a robust caspase-driven apoptosis in PC-3 cells. To gain a better understanding we will conduct an EGF/PDGR signaling array in which we will screen 84 genes and investigate the mechanism in which these actions may occur. Lastly we will validate gene expression by Real-Time PCR.

EVALUATION OF NON-*AGROBACTERIUM TUMEFACIENS*-MEDIATED TRANSFORMATION IN THREE PLANT MODELS: TOBACCO, ARABIDOPSIS AND WHEAT

Trainee: Lakisha Odom
Faculty Trainers: Ewen Mullins
Campus: Teagasc, Oak Park

LaKisha Odom*, Ewen Mullins, and Tony Wendt
Department of Agriculture and Environmental Science, Tuskegee University, AL, Department of Crop Science, Teagasc Oak Park Research Center, Carlow, IE.

Due to its wide-spread use and efficacy, it is thought that *Agrobacterium* is the only bacterial genus which has the ability to transfer DNA. However, according to Broothaerts et al. 2005, there are several species of bacteria outside of the *Agrobacterium* species that can be modified for gene transfer to a diverse number of plants. Previous research conducted by Wednt et al 2010 (unpublished data) demonstrated that "select *Rhizobia* spp. present an alternative technology to *A. tumefacien*s-mediated transformation systems for *S. tuberosum"* ; as well as high efficiency of transformation of *Arabidopsis* using floral dip and *N. Tabacum* was also transformed and showed stable integration of transgenes. The objective of this research was three-fold: to utilize additional isolates from the *Rhizobia* spp. armed with the same vector originally used previous research to determine if the capability of genetic transfer is limited to one bacterial strain or whether additional species are capable of genetic transfer; evaluate the efficacy of a member of the *Rhizobia* spp. designed with a vector which lacks the vir genes, in order to evaluate the presence of a natural mechanism involved in gene transfer; and evaluate the versatility of the *Rhizobia* spp. to transform a plant model which is not easily susceptible to *Agrobacterium* mediated transfer models or ATMT(i.e. wheat).

DEVELOPMENT OF TRANSGENIC SWEETPOTATO [I*pomoea Batatas (L Lam)] EXPRESSING SYNTHETIC LYTIC PEPTIDE GENES Jc41n AND Jc41nd AS A PLANT-BASED TREATMENT REGIMEN AGAINST HIV REPLICATION*

Trainee: Steven Samuels
Faculty Trainer: Marceline Egnin
Campus: Tuskegee University

S. Samuels*, M. Egnin, and J. Jaynes
Department of Agricultural and Environmental Sciences, Tuskegee University, AL 36088

Epidemic diseases such as AIDS caused by human immunodeficiency (HIV) virus are responsible for millions of deaths annually. CDC estimated in 2010, there are 1.2 million people infected in the US, especially within the Black Belt region which holds the highest HIV rates in rural America. Many epidemic diseases are now being fought by a new revolution of plant-based therapeutic treatments, which greatly reduce production processes such as posttranslational modifications, and synthesis of traditional modes of administration. Based plant transformation technology, synthetic lytic antiviral peptides JC41N and JC41ND, which are capable of inhibiting HIV progression have been developed at Tuskegee University. To produce these peptides in *planta*, two *de novo* synthetic gene constructs were designed to facilitate cloning in bacteria and accumulation in plants without lethality. Gene constructs of *jc41N* and *jc41ND* were cloned into the T-DNA borders of binary plasmid, pGPTV-kan, containing a kanamycin resistance gene. *Escherichia coli (E. coli)* DH5α cells were transformed with recombinant plasmid pGPTV/*jc41N* and pGPTV/*jc41ND*. Recombinant plasmids were mobilized in disarmed *Agrobacterium tumefaciens* strain EHA105. PCR confirmed *Agrobacterium* strains utilized in sweetpotato transformation. Through three transformation events, jc41N generated embryos with frequencies ranging from 16% to 45%, and *jc41ND* ranging from 4% to 58%. Optimal regeneration time varied among events ranging from 20 to 50 days on Embryo Production media. Fifty-five (55) kanamycin resistance embryos of D-3 transformed with *jc41N* and jc*41ND* genes were obtained and germinated on MM supplemented with Timetin 100mg/l and Kanamycin 12.5mg/l, resulting in twenty four (24) putative transgenic plantlets.

IDENTIFYING THE BIOCHEMICAL AND MOLECULAR COMPONENTS OF PLANT STRESS PERCEPTION

Trainee: Geoffrey Benn
Faculty Trainer: Katayoon Dehesh
Campus: UC Davis

Geoffrey Benn[1*], Marta Bjornson[1,2*], and Katayoon Dehesh[1]
1. Department of Plant Biology, UC Davis.
2. Department of Plant Sciences, UC Davis.

As sessile organisms, plants are unable to flee abiotic and biotic stresses in their environment. Therefore, the perception of stress and the subsequent initiation of appropriate responses are critical for plants. The physiological responses of plants to a variety of stresses have been intensely studied. However, the molecular and biochemical basis of the perception event leading to the initiation of these stress responses is poorly understood. Previous work in our lab using *Arabidopsis* identified a cis-element, the rapid stress response element (RSRE), which is sufficient to confer a rapid and transient transcriptional response to biotic and abiotic stressors, including cold, wounding, and pathogen attack. The goal of my research is to identify the molecular and biochemical basis for stress-induced up-regulation of RSRE-containing genes. I have shown that RSRE-containing genes are upstream of plant hormones involved in defense responses and have established a possible role for superoxide signaling in stress perception. To identify transcription factors that bind the RSRE, we will use a yeast one-hybrid screen in parallel with an affinity purification approach. Putative RSRE-binding factors will be further characterized using T-DNA knockout lines and ChIP-Seq.

PRODUCTION OF CHIMERIC ANTIMICROBIAL PROTEINS IN *PLANTA*

Trainee: Marta Bjornson
Faculty Trainer: Abhaya Dandekar, Katayoon Dehesh
Campus: UC Davis

Marta Bjornson*, Hossein Gouran, Camille Roux, and Abhaya Dandekar
Department of Plant Sciences, UC Davis.

Bacterial pathogens are a major limiting factor to agricultural yields worldwide. Next-generation chimeric antimicrobial proteins can relieve this pressure and increase yields. One such protein, a chimera consisting of a domain similar to Human Neutrophil Elastase and a domain similar to Cecropin B (HNE-Cec) confers varying degrees of resistance to Pierce's disease in transgenic grape plants. I have made a system to quickly generate large amounts of this and other proteins. In the short term this is useful to facilitate further study of the HNE-Cec protein. In the future it will be useful to produce related proteins for screening effectiveness against various bacterial pathogens. This system consists of *Nicotiana benthamiana* plants grown hydroponically for simplified recovery after transformation, the Tobacco Mosaic virus RNA-Based Overexpression (TRBO) vector in *Agrobacterium tumefaciens*, and a specialized vacuum chamber for infiltration. I have also tested several protein extraction and quantification techniques, including SDS-PAGE, Western blotting, and ELISA. The ability to produce large amounts of a desired protein for study *in planta* will facilitate studies of the mechanism of action of the HNE-Cec chimeric protein as well as development of more proteins targeted to new domains and bacteria.

QUANTITATIVE PROTEOMICS REVEALS DYNAMIC CHANGES AT THE PLASMA MEMBRANE DURING PLANT IMMUNE RESPONSES

Trainee: J. Mitch Elmore
Faculty Trainer: Gitta Coaker
Campus: UC Davis

J. Mitch Elmore[1*], Jun Liu[1], Brett Phinney[2], and Gitta Coaker[1]
[1]Department of Plant Pathology, University of California, Davis, CA
[2]Genome Center Proteomics Core Facility, University of California, Davis, CA

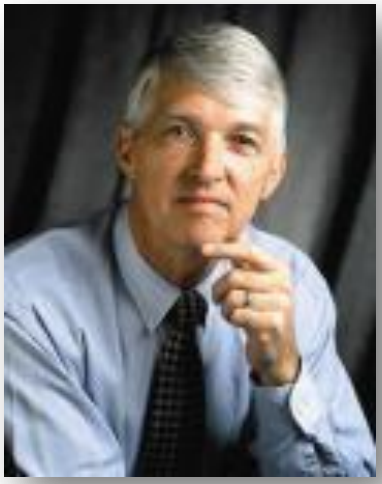
Innovative strategies for sustainable disease control in agriculture can be developed by understanding the mechanisms underlying plant-pathogen interactions. Over the last 20 years, much progress has been made to identify the plant disease Resistance (R) proteins responsible for pathogen recognition, but deciphering the molecular events following R protein activation has been a challenge. Our laboratory seeks to understand how the plant cellular proteome changes in order to mount effective defense responses. Because many classes of plant pathogens remain extracellular during their lifecycle, the plant plasma membrane mediates critical aspects of plant immunity. We have used quantitative proteomics to investigate how the plasma membrane proteome changes during plant immune signaling activated by the *Arabidopsis* R protein RPS2. Gel-enhanced liquid chromatography coupled with tandem mass spectrometry (GE LC-MS/MS) identified over 2300 proteins across 3 biological replicates. Approximately 20% of identified proteins significantly changed during activation of plant immunity when compared to controls. Proteins that are up-regulated at the plasma membrane during activation of plant defense responses include proteins involved in membrane scaffolding and transport, signal transduction, primary and secondary metabolism, and known regulators of plant immune signaling. These experiments highlight the dynamic nature of the plasma membrane proteome during plant defense responses. In addition, this research should identify novel components of plant immunity and targets for plant biotechnology to improve crop disease resistance.

INVESTIGATING THE IMPORTANCE OF NATURAL VARIATION IN THE GLUCOSINOLATE PATHWAY USING ARABIDOPSIS THALIANA

Trainee: Rachel Kerwin
Faculty Trainer: Dan Kliebenstein
Campus: UC Davis

Rachel Kerwin* and Dan Kliebenstein
Department of Plant Sciences, University of California, Davis, CA

Glucosinolates (GLS) are a class of secondary metabolites found in leaf tissue and seeds of plants from the order Brassicales, of which *Arabidopsis thaliana* is a member. These metabolites are natural pesticides that are activated upon herbivore attack (e.g. a caterpillar chewing on the leaves of the plant). In nature, there are hundreds of accessions of *Arabidopsis thaliana* that live in different environments and all produce these compounds. Due to natural variation in genes encoding transcription factors and biosynthetic enzymes, wild accessions of *Arabidopsis thaliana* show significant intraspecific variation in GLS phenotypes. However, analysis of ~300 wild accessions suggests that natural populations contain only a subset of the potential GLS genotypes that can be generated in the laboratory by crossing these plants (Kliebenstein et al., 2001; Lambrix et al., 2001; and unpublished data). This suggests that there is selection for and against specific phenotypes that restricts which genotypes survive and persist in wild populations. In addition, variation in GLS profiles is also likely being selected for due to diversity in herbivore communities. In order to test these hypotheses, I am creating an *Arabidopsis* population representing the majority of potential aliphatic GLS variation by making crosses between mutants in the accession Columbia-0 (Col-0). By using mutants that mimic natural polymorphisms (mutations) present within 9 genes representing 8 loci involved in glucosinolate diversity, a population expressing the different combinations of alleles in this metabolic pathway will be generated. This population will then be used to directly test the proposed hypotheses.

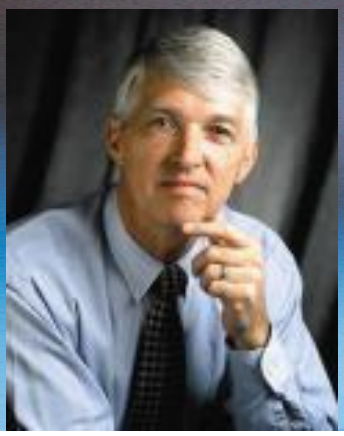


Distinguished Lecture

NSF CREATE-IGERT
2010-2011 Distinguished Lecture

Dr. Roger Beachy
Director, National Institute of Food & Agriculture

"Opportunities & Challenges in Agriculture and Biotechnology: Who Will Predict the Future?"



Tuesday, January 11, 2011
1005 Genome & Biomedical Sciences Facility, UC Davis
4:10 – 5:00 pm

The UC Davis NSF CREATE-IGERT program is honored to host Dr. Roger Beachy, the inaugural director of the National Institute of Food & Agriculture. As Director of NIFA, he oversees awarding extramural funds for Research, Extension and Education. Prior to joining USDA he served as the founding president of the Donald Danforth Plant Science Center in St. Louis, Missouri. In this role, Dr. Beachy was responsible for developing and implementing the Danforth Center's strategic direction and formulating its research programs. Dr. Roger N. Beachy, a member of the National Academy of Sciences, is internationally known for his groundbreaking research on developing virus-resistant plants based on pathogen derived resistance, including use of capsid protein sequences and other strategies that restrict virus infection and disease.

Prior to his role at the Danforth Center, Dr. Beachy headed the Division of Plant Biology at The Scripps Research Institute and co-directed the International Laboratory for Tropical Agricultural Biotechnology (ILTAB). He began his academic career at Washington University in St. Louis. In addition to membership in the National Academy of Sciences he has received numerous other awards including the 2001 Wolf Prize in Agriculture and is a fellow in the American Association for the Advancement of Science, the American Academy of Microbiology, and the National Academy of Science, India.

CREATE IGERT
COLLABORATIVE RESEARCH & EDUCATION IN AGRICULTURAL TECHNOLOGIES & ENGINEERING

Poster Session

Focus Area: Plant-Made Products

USING A TRBO-BASED PLANT EXPRESSION SYSTEM TO *ACCELERATE* INTESTINAL EPITHELIAL REGENERATION

Trainee: Tiffany Glavan
Faculty trainer: Satya Dandekar
Campus: UC Davis

Tiffany W Glavan[1*], Sang-Kyu Jung[2], Ben Lindenmuth[2], Larry Joh[2], Satya Dandekar[1], Abhaya Dandekar[3], and Karen McDonald[2]
[1]Department of Medical Microbiology and Immunology, University of California, Davis, CA, 95616
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Cells that comprise the intestinal epithelium are continually differentiating and migrating upward into the villi. Regeneration and renewal of this single cell layer is an ongoing process that is integral to gastrointestinal health and immune function. We are interested in developing a plant-based therapeutic aimed at accelerating mucosal regeneration and repair mechanisms, thus increasing barrier function in the gastrointestinal tract. The mitogenic protein of interest is R-spondin1, a growth factor known to positively regulate cell signaling in the Wnt pathway. As a therapeutic, this protein has the potential to provide a restoration of function in the context of multiple disease states that disrupt the intestinal epithelium, including radiation and chemotherapy-induced mucositis, inflammatory bowel disease, and human immunodeficiency virus infection.

A multidisciplinary collaborative effort is in place to develop an *Agrobacterium*-based plant expression system to produce this protein in tobacco plants. The human gene for R-spondin1 has been re-designed for expression in *N.benthamiana* through codon optimization and the addition of Kozak's context sequence, a six-histidine tag, and mRNA secondary structure aimed at decreasing degradation. This synthetic gene construct was inserted into a tobacco mosaic virus RNA-based overexpression (TRBO) vector, which was then electroporated into *Agrobacteria*. Immunodotblot analysis revealed positive expression of R-spondin1 in crude plant extracts and qRT-PCR was used to demonstrate increasing RNA levels. Anion exchange and metal affinity chromatography were used to purify R-spondin1. We plan to investigate the bioactivity of plant-derived R-spondin1 using a β-catenin stabilization assay on treated HEK293 cells.

Focus Area: Plant-Made Products

DEVELOPMENT OF A PROCESS FOR CYCLODEXTRIN PRODUCTION FROM SWEETPOTATO [*Ipomoea batatas (L.)Lam*.]

Trainee: Sonni-Ali Miller
Faculty Trainer: Jesse Jaynes
Campus: Tuskegee University

Sonni-Ali Miller* and Jesse Jaynes
College of Agricultural, Environmental, and Natural Science, Tuskegee University, AL

Translational studies using animal models in biomedical research are common with mice and rats being the most widely used models. When performing these studies maintenance of humane conditions and animal health are paramount to the accuracy of the data collected. Among these conditions is the reduction of pain in experimental subjects to the minimal amount necessary for the specific study. One method of pain reduction among experimental subjects may be use of alternative methods to administer treatments that are typically injected. Cyclodextrins may provide a potential alternative to typical intradermal injection. Cyclodextrins are produced from a relatively simple process that involves two-step enzymatic conversion of starch using α-amylase and CGTase.

The sweetpotato [*Ipomoea batatas (L.)Lam*.] provides a potentially strong candidate crop for starch isolation and enzymatic conversion into cyclodextrins with 70% of its dry matter being starch. Previous research at Tuskegee University has elucidated a method of starch isolation and syrup production from sweetpotato which will serve as a starting point for the cyclodextrin process. We are interested in evaluating the efficiency and feasibility of cyclodextrin production from sweetpotato as a means of developing a loading mechanism for drug administration and increasing potential uses for the crop. We will determine the most efficient manner of cyclodextrin production and then test its loading efficiency using a variety of small peptides as treatments in vivo.

Focus Area: Plant-Made Products

A RAPID, *IN-SITU* ASSAY FOR MEASURING *AGROBACTERIUM TUMEFACIENS* ATTACHEMENT TO LEAF TISSUE AS AN INDICATOR OF TRANSFORMATION EFFICIENCY

Trainee: Chris Simmons
Trainer: Jean VanderGheynst
Campus: UC Davis

Chris Simmons[1*], N. Nitin[1,2], and Jean VanderGheynst[1]
1. Department of Biological and Agricultural Engineering, University of California, Davis;
2. Department of Food Science and Technology, University of California, Davis

A. tumefaciens' ability to transform leaves varies greatly between different bacterial strains, leaf tissues and host plant species. The magnitude of *A. tumefaciens* binding to leaf cells immediately after exposure provides a potential early indicator of transformation efficiency across plant species and tissues within leaves. Imaging of bound *A. tumefaciens* labeled with fluorescent nucleic acid stain is a rapid alternative to creating recombinant strains expressing fluorescent proteins. In this study, *A. tumefaciens* was tagged with Syto 16 fluorescent nucleic acid stain and examined for the ability to divide and transfer transgenes to leaf tissue. No significant difference in viability and virulence was observed between stained and unstained bacteria. Confocal fluorescent microscopy was used to quantify bound *A. tumefaciens* in sections of lettuce and switchgrass leaf tissue immediately following vacuum-infiltration with labeled bacteria. Relative densities of bound bacteria within each host were in agreement with known differences in competency between these plants with respect to the particular strain of *A. tumefaciens* used. Together, nucleic acid staining of *A. tumefaciens* followed by confocal microscopy of infected leaf tissue offers a rapid, *in situ* method for evaluating relative avidity of *A. tumefaciens*' to various plant expression hosts.

Focus Area: Biofuels & Biorefineries

LOCALIZATION AND RECOVERY OF IN PLANTA PRODUCED CELLULASE ENZYME

Student: Nathaniel J. Kingsbury
Faculty Adviser: Karen McDonald
Campus: UC Davis

Nathaniel J. Kingsbury, Grace Bai, and Karen McDonald
Department of Chemical Engineering & Materials Science, University of California, Davis

In planta production of apoplast-targeted cellulose degrading enzymes may be a valuable approach either for in-situ decomposition of cellulose into sugars that can then be microbially fermented into advanced biofuels or for efficient recovery of concentrated enzyme preparations that are free from components that may inhibit biological conversion of other feedstocks. A vacuum infiltration-centrifugation method is being developed for obtaining apoplast wash fluid that removes the *Acidothermus cellulyticus* thermostable endoglucanase E1 that is transiently expressed in tobacco leaves. Using this method we are able to recover active E1 produced and found that the vast majority of the E1 removed was indeed localized to the apoplast. For optimal recovery of endoglucanase activity in the apoplast wash fluid, it may be necessary to add components to the infiltration buffer that will release E1 bound to the plant cell walls and increase cell wall porosity.

Focus Area: Biofuels & Biorefineries

RECHANNELING STARCH TO OIL

Trainee: Mark Lemos
Faculty Trainer: Katayoon Dehesh
Campus: Plant Biology Department, UC Davis

Mark Lemos*, Natasha Worden, Daniel Hayden, and Katayoon Dehesh
Department of Plant Biology, University of California Davis

In 2007, global liquid fuel consumption was 86.1 million barrels per day and is growing steadily towards the projected 110.6 million barrels per day by 2035. Growing economies such as China and India are rapidly increasing transportation fuel demands, and high energy consuming countries like the United States have not yet reached a plateau in their fuel needs. There is an undisputed need for the development of renewable energy and fuel sources which address the issues of increased need, rising levels of carbon dioxide, economic viability, and domestic energy security.

Biofuels offer a renewable source of liquid fuel. Oil based biofuels offer favorable economic and energy content over starch to ethanol produced biofuels. The goal of the project is to identify the regulatory genetic switches that determine the carbon flux to starch and oil biosynthetic pathways, with the ultimate goal of re-directing the reduced carbon to oil biosynthesis in plants. Using global transcriptomic analyses platform obtained from oil accumulating endosperms of two closely related oat cultivars, one a low-oil containing variety Freja and the other a high-oil containing variety Matilda, a number of candidate genes with potential key regulatory function in enhancing the carbon flux towards oil biosynthesis were identified. Several full length cDNAs for these candidates' genes have been cloned using Rapid Amplification of cDNA Ends (RACE). These cDNAs will be overexpressed and/or downregulated in *Arabidopsis thaliana* to determine their impacts on rechanneling of starch to oil. Towards this goal, we have transformed Agrobacterium GV3101 with a selected group of BASTA resistant destination vectors containing these cDNAs. Thus far, Agrobacterium-mediated transformation has been employed to introduce three of these cDNA containing constructs into *Arabidopsis.* Currently, we are screening the first generations of these transgenic plants using a combination of Basta selection and PCR analyses.

Focus Area: Biofuels & Biorefineries

TRANSIENT *IN PLANTA* EXPRESSION OF A CELLULOSE-DEGRADING ENZYME

Trainee: Benjamin E. Lindenmuth
Trainer: Karen McDonald
Campus: UC Davis

Benjamin E. Lindenmuth* and Karen McDonald
Department of Chemical Engineering and Materials Science, University of California, Davis.

Biofuels such as ethanol are fermented from glucose, and the cellulose in biomass is a potential source of this sugar. Large quantities of low-cost enzymes are needed to degrade the cellulose into glucose. In this project, leaves harvested from *Nicotiana benthamiana* plants are infiltrated with recombinant *Agrobacterium tumefaciens* to produce one of these enzymes. These bacteria carry the gene encoding endoglucanase from *Acidothermus cellulolyticus*, which is transferred to the host plant and expressed transiently.

Various buffers for extracting the endoglucanase from the plant tissue have been explored. Enzyme activity is measured by cleavage of a fluorescent substrate. A recombinant endoglucanase standard has also been produced in *Pichia pastoris*. These techniques can be applied to production of other enzymes in the synergistic set required for cellulose hydrolysis.

Focus Area: Biofuels & Biorefineries

INVESTIGATING THE ORIGIN AND COMPOSITION OF NANOPARTICLES DURING IMAGING OF CELLULOSE MICROFIBRILS ATOMIC FORCE MICROSCOPY.

Trainee: Patrick O'Dell
Faculty Trainer: Tina Jeoh
Campus: UC Davis

Patrick O'Dell*, Monica Santa-Maria, and Tina Jeoh
Department of Biological and Agricultural Engineering, University of California Davis

Lignocellulosic materials compose the large source of plant biomass on earth, and cellulose has been proposed as a feedstock for renewable biofuels. However, fuels from lignocellulosic biomass has not been commercialized for a number of reasons. Currently, the use of naturally occurring enzymes from fungus have been used to decompose cellulose, however the mechanism of action of these enzymes is not well understood. Our laboratory uses atomic force microscopy (AFM) to study the interaction between cellulose-degrading enzymes (cellulases) and cellulose microfibrils. In a recent study, we showed that cellulose microfibrils subjected to high energy ultrasonication form spherical aggregates with diameters in the range of 40 – 120 nm. Moreover, these nanoparticles were observed to be resistant to hydrolysis by purified the cellulose degrading enzyme, *Trichoderma reesei* Cel7A (TrCe7A). The study focuses on identifying both the composition of these nanoparticles and the factors leading to nanoparticle formation. In addition, this study will refine the procedure for preparing cellulose microfibrils for AFM imaging

Understanding the effects of pretreatments on the molecular structure of lignocellulosic biomass will increase the fraction of biomass that can successfully be converted into biofuel. In addition, a better understanding of the mechanisms behind enzymatic hydrolysis of lignocellulosic materials will lead to improvements in the saccharification process.

Focus Area: Biofuels & Biorefineries

OPTIMIZING ENERGY BEET UTILIZATION FOR PRODUCTION OF BIOFUELS AND COPRODUCTS

Trainee: Steven Zicari
Faculty Trainer: Ruihong Zhang
Campus: UC Davis

Steven Zicari* and Ruihong Zhang -
Department of Biological and Agricultural Engineering, UC Davis

Energy Beets, non-food sugar beets customized for energy production, are an attractive feedstock for renewable biofuel production in California, although thorough technical, economic, and environmental assessments have not yet been completed. Optimized Energy Beet production, processing, and fuel conversion methods may deviate significantly from existing sugar beet refining or fuel ethanol conversion schemes. Utilization of the whole beet (roots and leaves) employing multiple conversion pathways, including fermentation and anaerobic digestion, as well as consolidation and optimization of traditional processing, extraction, and fermentation operations are expected to increase project technical, financial, and environmental efficiencies. This research will serve to better characterize Energy Beet non-sucrose compositions, study their affects on conversion to fuels, and optimize downstream processing steps. Opportunities for upstream genetic plant modifications will also be identified. Immediate objectives include, firstly, identifying lower cost methods for extracting and storing beet juice and pulp and, secondly, maximizing fermentative bioethanol or biobutanol production and coproduct recoveries from both sucrose and non-sucrose components. Recombinant *Saccharomyces cerevisiae* and *Escherichia coli KO11* organisms and consolidated fermentation schemes will be investigated. Utilization of the whole beet, including both the roots and greens (leaves), will be investigated in the framework of an integrated biorefinery model.

Focus Area: Environmental Sustainability

INVESTIGATING GENERAL STRESS-RESPONSE NETWORKS IN ARABIDOPSIS

Trainee: Marta Bjornson
Faculty Trainer: Abhaya Dandekar, Katayoon Dehesh
Campus: UC Davis

Marta Bjornson[1,2*] and Katayoon Dehesh[2]
1. Department of Plant Sciences, UC Davis.
2. Department of Plant Biology, UC Davis.

Plants are subjected to a myriad of environmental stresses both biotic and abiotic in nature. Although plant responses to these stresses have been studied, the mechanisms of stress perception and induction of stress response have remained elusive. In microarrays conducted after wounding and after *Phytophthora* infection, abiotic and biotic stresses respectively, the expression levels and patterns of many genes are altered. Among these are four genes that are up-regulated under both conditions. Based on additional bioinformatics analysis, these genes are co-expressed, suggesting they belong to the same or closely related plant stress- responsive signaling networks. Two of these stress-responsive genes encode CAF1a and CAF1b, enzymes belonging to a family of deadenylases previously studied in Dehesh lab. Transgenic lines with altered expression of these two genes are available in the lab. The other two genes are an ethylene response factor and a gene of unknown function. I have generated transgenic plants that either enhance or reduce the expression of these two genes. In order to complete a genetic toolbox for studying this stress response network I will generate higher order mutants, silenced in multiple genes, and transgenic plants which overexpress multiple genes in this network. The response of these singly and combinatorially genetically modified plants to various stresses will clarify the role of these genes within the plant stress perception and signal transduction network.

Focus Area: Environmental Sustainability

UTILIZING A POLYPHENOLIC OXIDASE ENZYME AS AN ANTIBIOSIS AGENT IN *MEDICAGO SATIVA* PEST MANAGEMENT.

Trainee: Timothy Butterfield
Faculty Trainer: Abhaya Dandekar
Campus: UC Davis

Timothy Butterfield[1*], Sandra Uratsu[1], Anna Maria Ibanez [1], Dan Putnam [1], Abhaya Dandekar [1]
[1]Department of Plant Sciences, University of California, Davis, CA

Alfafa *(Medicago sativa*) is the third most valuable legal crop produced in California, directly contributing more than $1 billion annually to the California economy (CFAITC, 2008). Alfalfa breeders have selected for resistance to many diseases, pathogens and pests; however, Alfalfa Weevil, the lepidopteran armyworm complex, and stem nematode have proven difficult to control through traditional breeding techniques. Consequently, weevils and worms inflict significant losses in yield and add to the production cost throught the application of pesticides; for nematodes, no effictive control agent has been identified. Previous research has demonstrated that many secondary metabolites - including tannins produced by polyphenolic oxidases (PPOs) - possess anti-nutritive and toxic effects in high pH environments. Excitingly, the gut of many insect larvae is maintained at an elevated pH and many larvae do not possess antioxidizing species to quench the radicals found in many phenolics. This feature of the insect larval gut has been exploited previously and antiherbivory protection conferred by transgenic PPO products in poplar (Wang and Constabel, 2004) and tomato (Manahil et a., 2008).

We have created transgenic *Medicago sativa* plants expressing walnut PPO under the control of a constitutive promoter (35S::*Jr*PPO). The resulting transformants (and untransformed controls) have been clonally propagated to generate populations, and transgenic PPO activity has been confirmed by a direct, quantitative polyphenol oxidase activity assay (Broothaerts et al. 2000). These populations will be used to study the efficacy of *Jr*PPO as a pest management tool in alfalfa. Alfalfa weevils grown in a cage with a single trifoliate leaf will be analyzed for herbivory, growth, development, and mortality rates. In order to to test the hypothesis that phenolics may disrupt the larval insect gut, we will use pH- and redox state-sensitive sensors to analyze pH and redox potential differences in the larval gut when fed untransformed and *Jr*PPO alfalfa. In order to identify the phenolic compound(s) possessing antiherbivory activity, we will perform standard assays to profile and compare the tannin content of control and transgenic individuals. Our working hypothesis is that *Jr*PPO expressed in alfalfa will be an effective agent to be employed in Integrated Pest Management schemes designed to reduce the financial and health-related burden associated with application of toxic pesticide sprays.

Focus Area: Environmental Sustainability

CONTROL TOOLS FOR CROP PATHOGEN VECTORS

Trainee: Elenor Castillo
Faculty Trainer: Florence Negre-Zakharov
Campus: UC Davis

Elenor Castillo*, Abhaya Dandekar and Florence Negre-Zakharov
Department of Plant Sciences, University of California, Davis

The goal of this project is to develop alternative methods to pesticide applications by using volatile compounds naturally produced by plants. Volatiles make up the natural bouquet of aroma in plants fruit and vegetables. They are more commonly associated with attracting insects to flowers for pollination, but are also involved in the plants innate immune system and can repel herbivores that feed on plants. The defense-related function of volatile compounds will be harnessed to design control strategies against the psyllid, *Diaphorina citri* (or Asian Citrus Psyllid) that carries the bacterial disease Huanglongbing (HLB), also known as citrus greening disease. This disease, caused by *Candidatus Liberibacter*, is extremely virulent and has spread to many areas in the United States. Currently there is no cure and infection of a citrus tree leads to death within two years. In Vietnam, small growers plant guava trees next to citrus trees to repel the psyllid and a recent characterization of the guava tree volatile profile indentified sulfur-containing compounds as the likely repellent (Rouseff et al., 2006). The sulfur volatiles dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were hypothesized to provide protection from the psyllid carrying the disease.

As of now, a bacterial gene responsible for DMS and DMDS biosynthesis has been introduced, (after codon optimization) into MicroTom tomato plants using *Agrobacterium*-mediated transformation. Fifteen transgenic lines are being characterized for their volatile profiles using solid-phase microextraction followed by gas chromatography and detection by chemiluminescence (Scarlata and Ebeler, 1999). Ten transgenic lines were found to produce sulfur volatiles at higher levels than control plants; analysis of the remaining lines is currently in progress. Furthermore, the repellent properties of sulfur volatile-producing transgenic plants will be assessed by using bio-insect assays, which will determine if the psyllid is repelled by the transgenic plant.

The ultimate goal is to create transgenic citrus rootstocks that will produce sulfur volatiles to repel the psyllid carrying HLB. Once developed and deployed, this system will be compatible with biocontrol and other integrated pest management (IPM) practices, and will allow small growers to successfully grow citrus in areas where the disease is endemic.

Focus Area: Environmental Sustainability

QUANTITATIVE PROTEOMICS REVEALS DYNAMIC CHANGES AT THE PLASMA MEMBRANE DURING PLANT IMMUNE RESPONSES

Trainee: J. Mitch Elmore
Faculty Trainer: Gitta Coaker
Campus: UC Davis

J. Mitch Elmore[1*], Jun Liu[1], Brett Phinney[2], and Gitta Coaker[1]
[1]Department of Plant Pathology, University of California, Davis, CA
[2]Genome Center Proteomics Core Facility, University of California, Davis, CA

Most classes of plant pathogens remain extracellular during their lifecycle. As a result, the plant plasma membrane mediates critical aspects of plant immunity including pathogen recognition, signal transduction, and downstream defense responses. Investigating how the plasma membrane proteome changes during these events will identify novel components of plant defense responses and lead to a better understanding of plant immune signaling. We have used quantitative proteomics to investigate plasma membrane dynamics during effector-triggered immunity (ETI).

Transgenic *Arabidopsis* plants expressing the bacterial effector AvrRpt2 under the control of a dexamethasone-inducible promoter were used to induce ETI. Expression of the AvrRpt2 protease results in RIN4 cleavage and activation of the Resistance (R) protein RPS2. Plasma membrane vesicles were isolated 6 hours post-Dex treatment and subjected to gel-enhanced liquid chromatography tandem mass spectrometry (GE LC-MS/MS) for protein identifications. The QSpec spectral counting program was used to quantify relative protein abundance between treatments. Approximately 2300 proteins were identified across 3 biological replicates and over 20% are significantly changing during ETI. Proteins that are up-regulated at the plasma membrane during ETI include proteins involved in membrane scaffolding and transport, signal transduction, primary and secondary metabolism, and known regulators of plant immune responses. These experiments highlight the dynamic nature of the plasma membrane proteome during plant defense responses.

Focus Area: Environmental Sustainability

MEDIATION OF HUANGLONGBING AND CITRUS VARIEGATED CHLOROSIS USING CHIMERIC ANTIMICROBIAL PROTEINS

Trainee: Hyrum Gillespie
Faculty Trainer: Dr. Abhaya M. Dandekar
Campus: UC Davis

Hyrum Gillespie[1*], Hossein F. Gouran[1], Ana M. Ibáñez[1], Sandie L. Uratsu[1], George E. Bruening[2], Cecilia Agüero[1], Goutam Gupta[3] and Abhaya M. Dandekar[1]
[1]Dept. of Plant Sciences, University of California, Davis
[2]Department of Plant Pathology, University of California, Davis
[3]Biosciences Division, Los Alamos National Laboratory

Huanglongbing (HLB), an extremely destructive vector born disease caused by *Candidatus* Liberibacter *asiaticus,* has been found in citrus groves throughout Florida. Citrus Variegated Chlorosis (CVC), caused by *Xylella fastidiosa (Xf)* which inhabits the xylem of sweet oranges, has had a devastating economic impact on citrus groves throughout Brazil. While as of yet these pathogens have not entered California, both of these pathogens are posed to cause vast losses to California growers similar to those experienced worldwide. A chimeric antimicrobial protein (CAP) was designed in our lab to specifically target *Xylella fastidiosa,* and confers marked plant resistance to *Xf*, causative agent of Pierce's disease in grape. Transgenic citrus trees expressing this CAP will be tested to determine resistance to CVC. Further, a new CAP using a HLB specific outer membrane protein will be developed and tested for effectiveness in transgenic rootstock.

Focus Area: Environmental Sustainability

EFFECT OF COTTON PLANT GENETICALLY MODIFIED WITH AN ANTIMICROBIAL SYNTHETIC PEPTIDE D4E1 ON SOIL MICROBIAL DIVERSITY AND ENZYME ACTIVITY

Trainee: LaKisha Odom
Faculty Trainers: Jesse Jaynes and Ramble Ankumah
Campus: Tuskegee University

LaKisha Odom*, Ramble O. Ankumah, Conrad Bonsi, Jeff Cary, Marceline Egnin, Jesse Jaynes, Desmond Mortley, Lanell Ogden, and Kanniah Rajasekaran
Department of Agriculture and Environmental Science, Tuskegee University, AL

Cotton seedling disease (CSD) causes the largest yield losses of any cotton disease in the U.S. In the state of Alabama alone, CSD is responsible for an average of 10-million dollars per year in revenue. In Alabama, the pathogens primarily responsible for CSD are *Rhizoctonia solani* and *Pythium spp.* which belong to the fungal classes *Basiodomycetes* and *Oomycetes,* respectively. Its economic importance, coupled with the fact that cotton has no known disease resistant cultivars to CSD, make it an ideal candidate for genetic modification to confer disease resistance. *D4E1* is a synthetic antimicrobial peptide which, when transformed into cotton, *in vitro* and *in planta,* confers broad spectrum antimicrobial and antifungal properties against many fungal classes, including *Basiodomycetes* and *Oomycetes.* The effectiveness of *D4E1* against these pathogens and its effect on soil biological processes has yet to be tested *in situ*. The efficacy of D4E1 in the field and its effects on soil enzyme and microbial diversity was tested over two consecutive growing seasons on test plots using a completely randomized design. Treatments were: a control with GUS reporter gene, a non-transgenic parent variety, or one of 3 isogenic lines of cotton seed transformed with *D4E1* (designated 357, 358, and 373). Evidence of disease symptoms was evaluated bu assesses disease symptoms by assigning severity of disease symptoms a numerical score and the soil was randomly sampled, composited and subjected to DNA extraction, pyrosequencing and phosphatase enzyme assays. Overall results show that introduction of *D4E1* resulted in a decrease in disease symptoms when compared to the control. Enzymatic Activity and Microbial diversity did show temporally-associated changes that were not treatment related.

Focus Area: Environmental Sustainability

ASSESSING THE EFFECTS OF LAND USE ON MICROBIAL DIVERSITY AND PHYLOGENY ACROSS A MIXED CULTURE AGROECOSYSTEM

Trainee: Raymon Shange
Faculty Trainer: Ramble Ankumah
Campus: Tuskegee University

Raymon S. Shange*, Ramble Ankumah, Leonard Githinji, and Robert Zabawa
Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL, 36088

Land-use change and management are normally enacted to manipulate environments to improve conditions that relate to production, remediation, and accommodation. The effects of these practices have been shown to have indirect, but profound effects on the communities of native organisms of the associated ecosystems. Though changes in soil microbial communities have been extensively studied, the depth of these inquiries was limited given the state of the research technology. Nonetheless, with the advent of next-generation sequencing, the opportunity to study ecological issues in microbial ecosystems has been greatly enhanced. In the present study, replicate soil samples were collected from a demonstration farm in which three land-use systems were exhibited (grazed pine forest, cultivated crop, and grazed pasture) on a single soil type in Perry County Alabama. Bacterial-tag encoded FLX amplicon pyrosequencing was used to generate genomic libraries targeting 16S rRNA. The different land use systems also showed distinction in the structure of their microbial communities with respect to the differences detected in cluster analysis as well as diversity indices. Specific taxa (most notably Actinobacteria, Acidobacteria, and classes of Proteobacteria) showed significant shifts across the land-use strata. Selected soil properties (SOM, soil texture, pH, and enzyme activity) also differed significantly across land-use systems, while showing variation consistent with changes in microbial phyla. Together these results suggest that pyrosequencing along with traditional analysis of physical and chemical soil properties may be able to provide insight into the biogeography of microbial communities across landscapes with respect to ecological disturbance.

Focus Area: Environmental Sustainability

TIMELY SEPTATION REQUIRES SNAD-DEPENDENT SPINDLE POLE BODY LOCALIZATION OF THE SEPTATION INITIATION NETWORK COMPONENTS IN THE FILAMENTOUS FUNGUS *ASPERGILLUS NIDULANS*

Trainee: Cui Jing (Tracy) Zeng
Faculty Trainer: Bo Liu
Campus: UC Davis

Cui Jing Tracy Zeng[1*], Jung-Mi Kim[1], Tania Nayak[2], Rongzhong Shao[1], Angel Huang[1], Berl R. Oakley[2], and Bo Liu[1]
[1]Department of Plant Biology, University of California, Davis, CA, 95616
[2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, 66045

The cell wall of filamentous fungi is not only an important structural component, but also plays a major role in sequestering toxic metals from the environment. The formation of cell walls during cytokinesis/septation also contributes to the robust growth of fungi. Interestingly, unlike other eukaryotes, filamentous fungi don't make cell walls after each round of nuclear division. The mechanisms that control the onset of cell wall formation remain to be elucidated. In the filamentous fungus *Aspergillus nidulans*, cytokinesis/septation is triggered by the kinase cascade of the septation initiation network (SIN) which first appears at the spindle pole body (SPB) during mitosis. The novel coiled-coil protein SNAD is associated with the SPB, and is required for timely septation, and conidiation. We have determined that SNAD acts as a scaffold protein that is required for the localization of the SIN proteins of SIDB and MOBA to the SPB. Another scaffold protein SEPK/SNAE, whose localization at SPB was dependent on SNAD, was also required for SIDB and MOBA localization to the SPB. In the absence of either SEPK/SNAE or SNAD, SIDB/MOBA successfully localized to the septation site, indicating that their earlier localization at SPB was not essential for their later appearance at the division site. Our results suggested that through SEPK/SNAE, SNAD mediates the interaction between SIN components and cell cycle regulators at the SPB. Unlike their functional counterparts in fission yeast, SEPK/SNAE and SNAD were not required for vegetative growth except for timely septation. Furthermore, hyperactivation of the SIN pathway by downregulation of negative regulators of the SIN suppressed the phenotype of aborted conidiation due to the loss of SNAD. Therefore, we conclude that SPB localization of SIN components is not essential for septation *per se*, but critical for septation to take place in a timely fashion. In addition, we conclude that timely execution of septation is a prerequisite for conidiation.

CREATE-IGERT

Trainee Biographies

CREATE-IGERT Trainees

Arzola, Lucas

McDonald Laboratory
Department of Chemical Engineering & Materials Science, Chemical Engineering Graduate Program, UC Davis

Research Focus: Plant-Made Products

The constant threat of bioterrorism and the recent H1N1 flu pandemic highlight the importance of developing the rapid, scalable, and cost-effective production of therapeutic agents. Recent advances in the field of plant biotechnology have made possible the use of plants as cost-effective biofactories of therapeutic proteins. Lucas' research focuses on the development of a plant based transient expression system in tobacco plants, for the production of an anthrax receptor decoy protein that can mitigate the effects of anthrax.

Benn, Geoff

Dehesh Laboratory
Department of Plant Biology, Plant Biology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Geoff is working on plant signaling and environmental stress responses. Understanding the underlying mechanisms of plant responses to environmental cues, both biotic and abiotic, will aid in engineering crop tolerance and increasing relative yields.

Bjornson, Marta

Dehesh Laboratory & Dandekar Laboratory
Department of Plant Biology and Department of Plant Sciences, Horticulture and Agronomy Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Marta is looking at the potential signaling role of arachidonic acid in eliciting plant-stress responses. Recently it has been demonstrated that this fatty acid modulates plant responses to a range of pathogens through alteration of jasmonic acid and salicylic acid stress responsive pathways. Marta's project will elucidate various components of arachidonic acid-mediated plant stress perception and response networks. Her findings have the potential of discovering novel strategies to enhance plant resistance to pests.

Butterfield, Timothy

Dandekar Laboratory,
Department of Plant Sciences, Plant Biology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Tim is pursuing research that will lead to new strategies for agronomic crop improvement in alfalfa. Specifically, his doctoral work will investigate whether increased Tannic acid and Gallic acid may function as Integrative Pest Management tools. We hypothesize that these metabolites confer herbivory deterrence against alfalfa weevil and army worms - the greatest insect pests in California agricultural fields. The findings of this research may result in reduced pesticide applications in California alfalfa fields - improving farm worker safety, safeguarding water supplies, and reducing the death of non-target insect species.

Castillo, Elenor

Negre-Zakharov Laboratory
Department of Plant Sciences, Plant Biology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Elenor's project focuses on elucidating the metabolic pathways that underlie production of aromatic volatiles in fruits, which has direct commercial application in extending fruit shelf-life. On a broader scale, understanding the role of volatile chemical signals within and between plants in field populations may also play a part in increasing crop yields/biomass, engineering insect and pathogen resistance, and fine-tuning other agronomic and quality-related crop traits.

Chiniquy, Dawn

Ronald Laboratory & Joint BioEnergy Institute (JBEI)
Department of Plant Pathology, Plant Biology Graduate Group, UC Davis

Research Focus: Biofuels & Biorefineries

Gaining a greater understanding of the enzymes that build the plant cell wall may lead to improved feedstocks that make a cheaper, more efficient biofuel. Dawn focuses on characterizing genes in rice that build the cell wall and whether these genes could be altered for an improved feedstock for biofuels.

Elmore, James Mitch

Coaker Laboratory
Department of Plant Pathology, Plant Biology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Innovative strategies for sustainable disease control in agriculture can be developed by understanding the molecular mechanisms underlying plant-pathogen interactions. Mitch's research seeks to identify the plant targets and virulence mechanisms of proteins essential to the lifestyle of phytopathogenic bacteria.

Gales, Dominique N.

IBS Program, Yates Laboratory
Department of Agriculture and Environmental Sciences, Tuskegee University

Research Focus: Plant-Made Products

Dominique's primary motives are to contribute to the field of oncology and natural agents. She is currently working on the development of natural agents such as Purslane (***Portulaca oleracea*)** for the drug treatment of Human Prostate cancer cells. This natural agent is a succulent plant found in the subtropical and Mediterranean areas. It is a rich source of polyphenols, vitamins, minerals, dietary fiber, and omega-3 fatty acids. These nutrients have been shown to have positive impact on the reduction of risk factors associated with cardiovascular diseases, and may be effective against proliferation of cancer cells.

Gillespie, Hyrum

Dandekar Laboratory
Department of Plant Sciences, Genetics Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Hyrum will be developing biomarkers for disease identification in vector-borne citrus diseases, Huanglongbing (HLB) and Citrus Variegated Chlorosis (CVC). In addition to developing robust methods of monitoring disease progression, he will engineer and express a chimeric antimicrobial protein (CAP) in transgenic citrus rootstocks with the goal of developing disease-resistant trees.

Glavan, Tiffany

Dandekar Laboratory
Department of Medical Microbiology & Immunology, Microbiology Graduate Group, UC Davis

Research Focus: Plant-Made Products

Tiffany's project is focused on the development of plant-derived therapeutic proteins to treat gastrointestinal dysfunction through the regeneration and renewal of the epithelial layer of the gut mucosa. She is collaborating with multiple groups on campus in an effort to express the protein in *N.benthamiana* and evaluate its activity in epithelial cell culture.

Kerwin, Rachel

Kliebenstein Laboratory
Department of Plant Sciences, Plant Biology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Rachel seeks to understand the metabolic pathways underlying glucosinolate production in plants. She will characterize nine genes involved in glucosinolate metabolism and investigate glucosinolate's potential as plant-made product to deter herbivory in the field.

Lateef, Dalya

IBS Program, Bovell-Benjamin Laboratory
College of Agricultural, Environmental, and Natural Sciences
College of Veterinary Medicine and Allied Health, Tuskegee University

Research Focus: Plant-Made Products

Dalya is looking at the role of the enteric nervous system in the short term control of food intake by cholecystokinin. Recent projects include the investigation of candidate genes in the development of holoprosencephaly (HPE). HPE is a brain malformation that is caused by incomplete cleavage of the prosencephalon.

Lemos, Mark

Dehesh Laboratory,
Department of Plant Biology, Plant Biology Graduate Group, UC Davis

Research Focus: Biofuels & Biorefineries

Mark will be modifying the expression of duckweed genes involved in starch-to-oil metabolic channeling with the goal of decoupling these two processes. Ultimately, Mark is interested in development of duckweed as a scalable biomass crop and as a system for the

production of biofuels and high value plant-made products, such as nutraceuticals and pharmaceuticals.

Lindenmuth, Ben

McDonald Laboratory
Department of Chemical Engineering & Materials Science, Chemical Engineering Graduate Program, UC Davis

Research Focus: Biofuels & Biorefineries

Ben's research is focused on the development of an inducible plant-based expression system for the production of cellulose degrading enzymes. He is investigating the expression and localization of these enzymes *in planta*, as well as their potential use in an exogenously applied biomass pre-treatments.

Miller, Sonni-Ali

IBS Program, Martinez Laboratory
College of Agricultural, Environmental, and Natural Sciences
College of Veterinary Medicine and Allied Health, Tuskegee University

Research Focus: Plant-Made Products

Sonni is interested in multi-faceted research concerning interactions between nutrition and cytological behavior, specifically in energy nutrient metabolism. He is characterizing specific biomarkers in atherosclerosis as a result of differences in lipid metabolism. He is also investigating the efficacy of peptide fragments as treatments effecting plaque formation in various rodent models.

O'Dell, Patrick

Jeoh Laboratory
Department of Biological & Agricultural Engineering, Biological Systems Engineering Graduate Program, UC Davis

Research Focus: Biofuels & Biorefineries

Patrick's work concerns the molecular interactions between cellulose and cellulose-hydrolyzing enzymes. This research will use multiple types of high resolution microscopy, including confocal microscopy and atomic force microscopy, to study the kinetic mechanisms of cellulose hydrolysis by cellulases.

Odom, LaKisha

IBS Program, Ankumah Soil and Water Quality Laboratory
College of Agricultural, Environmental and Natural Sciences & College of Veterinary, Nursing, and Allied Health, Tuskegee University.

Research Focus: Environmental Sustainability

Evaluating through field trials, the efficacy of a transgenic cotton plant which has been transformed with a synthetic antimicrobial peptide, *D4E1,* on the progression of cotton seedling disease, soil microbial diversity, and enzymatic activity.

Samuels, Steven B.

IBS Program, Egnin Laboratory
College of Agricultural, Environmental and Natural Sciences, Tuskegee University

Research Focus: Plant-Made Products

Steven is working on the development of transgenic sweetpotato lines expressing synthetic lytic peptides, for potential therapeutic uses. In addition to developing transgenic plants for the biomanufacture of drugs and vaccines in developing countries, Steven is interested in the use of transgenic plants to increase yields and nutrient levels of staple crops.

Shange, Raymon S.

IBS Program, Ankumah Soil and Water Quality Laboratory & Zabawa Laboratory
College of Agricultural, Environmental, and Natural Sciences,
Tuskegee University

Research Focus: Environmental Sustainability

Raymon's research has directed him into the area of metagenomics and bioinformatics. His future concerns in research include the characterizations of microbial populations in differing environments, and their transcriptomic and proteomic responses to human influence (natural resources communities as well as organismal mutualisms). He retains an avid interest in the interface between science, the humanities, and society and hopes to be able to venture into this area in the future as well. He maintains his interests and completes lectures in Environmental Justice, Environmental Sustainability, Environmental Ethics, and the Philosophy of Nature.

Simmons, Chris

VanderGheynst Laboratory
Department of Biological & Agricultural Engineering, Biological Systems Engineering Graduate Program, UC Davis

Research Focus: Plant-Made Products

Efficient, high-level transformation of leaf tissues is required for utilizing harvested plant tissue as an expression host for the production of recombinant proteins. Chris's research seeks understand the fate of the plant-transforming pathogen *Agrobacterium tumefaciens* once it enters harvested leaf tissue with the ultimate goal of improving plant transformation efficiency through manipulation of variables affecting bacteria/plant cell interactions.

Wolf, Mark

Parales Laboratory
Department of Microbiology, Biochemistry & Molecular Biology Graduate Group, UC Davis

Research Focus: Biofuels & Biorefineries

Mark will characterize a number of lignocellulose-degrading enzymes isolated from *Acidothermus cellulolyticus*, a hot springs microorganism isolated in Yellowstone National Park. His goal is to identify thermostable enzymes appropriate for use in the production of biofuels and industrial chemicals.

Wu, Phoebe

Fan Laboratory
Microbiology Graduate Group, UC Davis

Research Focus: Biofuels & Biorefineries

Phoebe's research aims to develop a novel biochemical platform which will consolidate the costly steps it normally takes to convert cellulosic biomass to organic fuels into one biological process using an engineered cellulolytic fungus *Neurospora crassa* as the model organism. If successful, this research can lead to a new technology which can lower the cost of cellulosic biomass processing drastically.

Zeng, Cui Jing (Tracy)

Liu Laboratory
Department of Plant Biology, Microbiology Graduate Group, UC Davis

Research Focus: Environmental Sustainability

Tracy will identify components that are important for triggering the onset of cell wall formation using *Aspergillus nidulans* as a model organism. The goal of her studies is to design novel approaches aimed at manipulating filamentous fungi better suited for applications like fermentation and bioremediation.

Zicari, Steve

Zhang Laboratory
Department of Biological and Agricultural Engineering, Biological Systems Graduate Group, UC Davis

Research Focus: Biofuels & Biorefineries

Steve's research aims to better characterize Energy Beet non-sucrose compositions, study their affects on conversion to fuels and optimize downstream processing steps. Opportunities for upstream genetic plant modifications will also be identified. Steve's research will be conducted as a larger collaborative UCD research effort lead by Dr. Ruihong Zhang aimed at developing advanced biomass and conversion systems for producing biofuels and coproducts with Energy Beets and saline tolerant crops as core feedstocks.