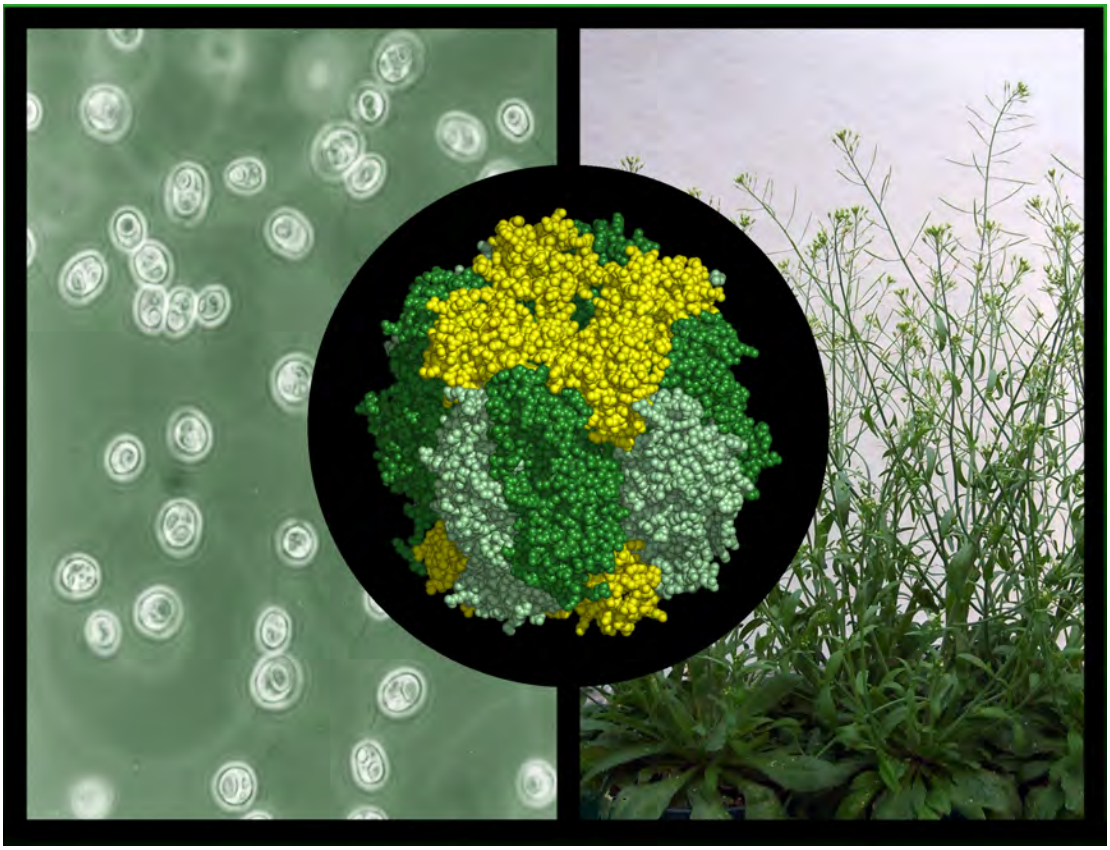
UNIVERSITY OF NEBRASKA—LINCOLN

INSTITUTE OF AGRICULTURE AND NATURAL RESOURCES

Annual Meeting of Multi-State Project NC-1200

Regulation of Photosynthetic Processes

November 23, 2013



Multi-State Meeting Schedule, Nebraska AES

NC-1200 Regulation of Photosynthetic Processes

Friday, November 22, 2013

6:30 PM Meet in the lobby of the Courtyard Marriott Hotel (808 R Street). Walk to Lazlo's for dinner (210 North 7th Street and P Street).

Saturday, November 23, 2013

7:00 AM Breakfast at your hotel (Courtyard Marriott).
8:30 AM Meet in hotel lobby. Hotel van departs for the Beadle Center (19th and Vine). Our meeting will be held in room E228.
9:05 AM Announcements and introductions, Julie Stone
9:15 AM Welcome from Dr. Archie Clutter, Dean and Director, Nebraska AES
9:30 AM Julie Stone, Nebraska AES
10:00 AM Bob Spreitzer, Nebraska AES
10:30 AM Don Weeks, Nebraska AES
11:00 AM Break
11:15 AM Tom Sharkey, Michigan AES
11:45 AM Tom Okita, Washington AES
12:15 AM Lunch in the atrium of the Beadle Center
1:30 PM Steve Huber, Illinois ARS
2:00 PM Karen Koch, Florida AES
2:30 PM JC Jang, Ohio AES
3:00 PM Break
3:15 PM Christoph Benning, Michigan AES
3:45 PM Rob Aiken, Kansas AES
4:15 PM Vara Prasad, Kansas AES
4:45 PM Felix Fritschi, Missouri AES
5:15 PM Break
5:30 PM Business meeting
6:30 PM Transportation departs for Courtyard Marriott Hotel.
7:45 PM Meet in hotel lobby and walk to the Cellar at the Oven (201 North 8th Street @ P Street).

Sunday, November 24, 2013

AM - PM Breakfast is served at the Courtyard Marriott Hotel. Other restaurants are within walking distance. Arrange hotel shuttle or taxi (402-477-6074) for transport to airport.

Participation List

NC-1200 Regulation of Photosynthetic Processes

Members Attending
Robert Aiken, Kansas State University
Christoph Benning, Michigan State University
Steve Huber, USDA, University of Illinois
Felix Fritschi, University of Missouri
Jyan-Chyun Jang, Ohio State University
Karen Koch, University of Florida
Thomas Okita, Washington State University
Vara Prasad, Kansas State University
Thomas Sharkey, Michigan State University
Robert J. Spreitzer, University of Nebraska
Julie M. Stone, University of Nebraska
Donald P. Weeks, University of Nebraska

Administrative Advisor
Christoph Benning, Michigan State University

Guests Attending
Raymond Chollet, University of Nebraska
Archie Clutter, University of Nebraska

Members Not Attending
Fred Below, University of Illinois
John Cushman, University of Nevada
Gerald Edwards, Washington State University
John Erwin, University of Minnesota (*no report provided*)
Laura Gentry, University of Illinois
Glenda Gillaspy, Virginia Tech
Michael Giroux, University of Montana
Jeff Harper, University of Nevada (*no report provided*)
David Kramer, Michigan State University (*no report provided*)
Jiaxu Li, Mississippi State University
Wayne Loescher, Michigan State University (*no report provided*)
Tasios Melis, University of California-Berkeley (*no report provided*)
James Moroney, Louisiana State University (*no report provided*)
Steven Rodermel, Iowa State University
Michael E. Salvucci, USDA-Arizona (*no report provided*)
Martin Spalding, Iowa State University (*no report provided*)

Steve Rodermel, Iowa State University, Iowa AES

Objective 1. Plastid Function and Intracellular Communication
Impact Statement:
PTOX plays an important role early in the process of thylakoid membrane biogenesis. Further insight into the function of this protein in photosynthesis, plant development and plant stress responses might lead to the design of strategies to manipulate the photosynthetic capacity and quality of important crop plants.

Accomplishments:
1. PTOX is a quinol terminal oxidase that participates in control of the redox poise of the plastoquinone pool, and it acts as a safety valve in high light conditions to shunt excess electrons from the PQ pool to oxygen, forming water. To gain insight into the function of PTOX, second- site suppressor analyses were performed. I
2. In this reporting period we found that *immutans* can be suppressed by *gigantea (gi*), a well-known late-flowering mutant. GI helps control the circadian clock. The *im gi* double mutant is late flowering, has reduced apical dominance, and is variegated during the early stages of development; however, during the later stages, newly emerging leaves are completely green. *im gi* also exhibits enhanced starch accumulation, likely due to enhanced rates of photosynthesis.
3. We found that suppression of variegation occurs at the floral transition and is mediated by an upregulation of cytokinins and a downregulation of GA. These analyses provide compelling evidence for a critical role of GI in modulating plastid biogenesis.

Plans for Coming Year:
- We will continue to characterize the *immutans* suppressor lines.

Publications:

Wang, M., Liu, X., Wang, R., Li, W., Rodermel, S., and Yu, F. (2012). Overexpression of a putative *Arabidopsis* BAHD acyltransferase causes dwarfism that can be rescued by brassinosteroid. J. Exp. Bot. **63:** 5787–5801.
Foudree, A., Putarjunan, A. Kambakam, S., Nolan, T., Fussell, J., Pogorelko, G., and Rodermel, S. (2012). The mechanism of variegation in *immutans* provides insight into chloroplast biogenesis. Front. Plant Physiol. **3:** Article 260.
Fu, A., Liu, H., Yu, F., Kambakam, S., Luan, S., and Rodermel, S. (2012). Alternative oxidases (AOX1a and AOX2) can functionally substitute for plastid terminal oxidase in *Arabidopsis* chloroplasts. Plant Cell **24:** 1579-1595.
Putarjunan, A., Liu, X., Nolan, T., Yu, F., and Rodermel, S. (2013). Understanding chloroplast biogenesis using second-site suppressors of *immutans* and *var2.* Photosynth. Res. **116:** 437–45.

Julie M. Stone, University of Nebraska, Nebraska AES

Objective 1: Plastid Function and Intracellular Communication
Impact Statement: Human DJ-1 has garnered great interest due to its implication in protecting cells against oxidative stress and consequently many different human diseases, including various cancers and neurodegenerative diseases. Little is known about the function of plant DJ-1-like proteins, but they are likely to mediate their functions in manners analogous to other superfamily members. A key structural feature of all DJ-1 superfamily members is that they form dimers (or higher oligomers) with differing interfaces, and these oligomers are essential for the function of these proteins. The unique gene structure of the plant DJ-1-like proteins strongly suggests that these plant proteins form a DJ-1-like "dimer" within a single polypeptide. If true, this will significantly simplify certain experiments, particularly the coimmunoprecipitation and/or yeast two-hybrid experiments to identify interacting macromolecules.

Accomplishments:
1. Using molecular genetics approaches, we have identified knockout mutants for some of the *A. thaliana* DJ-1 homologs. At least one gene is essential for viability. Two *AtDJ1C* gene T-DNA insertion mutant alleles confer an albino, seedling-lethal phenotype. We've also demonstrated that the
2. Wild-type DJ1C protein is targeted to chloroplasts and mutant, albino plant tissues (cotyledons) have severely disrupted chloroplast ultrastructure indicating that the protein normally functions in chloroplast biogenesis.
3. The chloroplast biogenesis defect associated with *dj1c* null mutations is effectively complemented with a functional epitope-tagged version of the protein (pDJ1C:gDJ1C:GFP).
4. AtDJ1C partial loss-of-function (RNAi) lines with varying levels of *DJ1C* expression (and varying levels of chloroplast dysfunction) have been generated.

Plans for Coming Year:
- We are attempting to complement *dj1c* with a construct with a expressing DJ1C-GFP under control of the constitutive 35S CaMV promoter to get sufficient material for co-immunoprecipitation experiments.
- We are attempting to complement *dj1c* with a similar construct harboring human DJ1.

Publications:
Kimberlin, A.N., Majumder, S., Han, G., Chen, M., Cahoon, R.E., Stone, J.M., Dunn, T.M., and Cahoon, E.B. (2013). Arabidopsis 56-amino acid serine palmitoyltransferase-interacting proteins stimulate sphingolipid synthesis, are essential, and affect mycotoxin sensitivity. *Plant Cell* (in press); doi: http://dx.doi.org/10.1105/tpc.113.116145.

Gerald Edwards and Tom Okita, Washington State University, Washington AES

Objective 2: Photosynthetic Capture and Photorespiratory Release of CO_2

Impact Statement:
Structural, biophysical and physiological traits were identified in rice and wild relatives in genus Oryza which control photosynthesis, transpiration and water use efficiency. Structural transitions were identified in succulent eudicots (family Chenopodiaceae) which show how species having a form of C4 photosynthesis evolved from C3 species.

Accomplishments:
1. In a study on the structural and functional traits of rice we provided insight into modifications which will be required to install C_4 photosynthesis; and, information on structural and functional traits in wild relatives which will be useful in employing strategies for increasing photosynthesis and water use efficiency in rice.
2. In a review, we discuss features of terrestrial and aquatic plants having single cell C_4 photosynthesis, consider the structure of rice mesophyll cells, and propose a form of C_4 cycle which could provide supplemental CO_2 to Rubisco in rice mesophyll chloroplasts.
3. In family Chenopodiaceae we identified structural transitions from C_3, to C_3-C_4 intermediates, to C_4 which shows how some succulent species develop a form of Kranz anatomy around the periphery of the leaf which surrounds all the water storage and vascular tissue.

Plans for Coming Year:
- Characterize the biochemical and structural transitions along a longitudinal leaf gradient which lead to the development of functional single-cell C_4 photosynthesis (family Chenopodiaceae).
- Characterize the biochemical and structural transitions along a longitudinal leaf gradient in eudicots which leads to the development of C_4 photosynthesis having different forms of Kranz anatomy.
- Determine the consequences of photorespiratory release of CO_2 in rice by studying the relationship between the kinetic properties of Rubisco, the CO_2 compensation point and refixation of photorespired CO_2.

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Impact Statement:
Structure-function relationship studies were conducted on the role of the rice endosperm ADPlgucose pyrophosphorylase (AGPase) in starch biosynthesis.

Accomplishments:
1. The catalytic activity of the major rice endosperm AGPase is controlled by redox potential via oxidation of cysteine residues on the large subunit.
2. Missense mutations in the large subunit of the major rice AGPase resulted in a 3- to 4-fold decrease in catalytic activity and a 4- to 6-fold decrease in the affinity for the activator 3-PGA.
3. Transcriptome analysis of transgenic rice plants over-producing starch indicate major changes in starch metabolism and a re-programming of metabolism.

Plans for Coming Year:
- Continue transcriptome analysis of over-producing starch plants and validate changes in gene expression.

- Identify the cysteine residues involved in redox control of the rice AGPase

Publications (2013):

Kirchhoff, H., Sharpe, R.M., Herbstova, M., Yarbrough, R., and Edwards, G.E. 2013 Differential mobility of pigment-protein complexes in granal and agranal thylakoid membranes of C_3 and C_4 plants. Plant Physiol **161,** 497-507.

Voznesenskaya, E.V., Koteyeva, N.K., Akhani, H., Roalson, E.H., and Edwards, G.E. Structural and physiological analyses in Salsoleae (Chenopodiaceae) indicate multiple transitions among C_3, intermediate and C_4 photosynthesis. J Exp Bot **64,** 3583-3604.

Giuliani, R., Koteyeva, N., Voznesenskaya, E., Evans, M.A., Cousins, A.B., and Edwards, G.E. Coordination of leaf photosynthesis, transpiration and structural traits in rice and wild relatives (genus *Oryza*). Plant Physiol **162,** 1632-1651.

Suleyman, A., Shen, J-R., and Edwards, G.E. 2013 Guest Editorial. Special issues on Photosynthesis Education honoring Govindjee. Photosyn Res **116**,107-110.

Ocampo, G., Koteyeva, N.K., Voznesenskaya, E.V., Edwards, G.E. Sage, T.L., Sage, R.F. and Columbus, J.T. Evolution of leaf anatomy and photosynthetic pathways in Portulacaceae. Am J Bot, In press.

Hwang S.-K,. Tuncel, A. and Okita, T.W. 2013. Redesigning starch metabolism to increase rice yields. In: Muralidharan K, Viraktamath BC and Siddiq EA, Eds. 2012. Proceedings of International Dialogue on Designer Rice for Future: Perception and Prospects, July 9-10, 2012. Society for Advancement of Rice Research, Directorate of Rice Research, Hyderabad, India.

Tuncel, A. and Okita, T.W. (2013) Improving starch yield in cereals by over-expression of ADPglucose pyrophosphorylase: Expectations and unanticipated outcomes. Plant Science, 211:52-60.

Wakuta, S., Nishimura, Y, Obana, Y., Saburi, W., Hamada, S., Ito, H., Hwang, S.-K., Okita, T.W., and Matsui, H. (2013) Modulation of allosteric regulation by E38K and G101N mutations in the potato tuber ADP-glucose pyrophosphorylase. Bioscience, Biotechnology and Biochemistry, 77:1854-1859.

Gardin, A., Koteyeva, N., Voznesenskaya, E., Edwards, G.E., and Cousins, A. The impact of growth temperature on photosynthesis and mitochondrial respiration in the C_3-C_4 intermediate *Salsola divaricata*. Plant Cell Environ, Submitted.

Rosnow, J. Yerramsetty, P.K., Berry, J.O., Okita, T.W., and Edwards, G.E. Exploring features linked to differentiation and formation of dimorphic chloroplasts in the single cell C_4 species *Bienertia sinuspersici*. BMC Plant Biol, Submitted

Rosnow J., Edwards, G.E., and Roalson, E.H. Evolutionary dynamics of phosphoenolpyruvate carboxylase in Kranz and non-Kranz C_4 Suaedoideae (Chenopodiaceae). J. Exp Bot, Submitted

Offermann, S., Friso, G., Doroshenk, K.A., Sun, Q., Sharpe, R.M., Okita, R.M., Okita, T.W., Edwards, G.E., and van Wijk, K.J. Development and subcellular organization of single-cell C_4 photosynthesis in *Bienertia sinuspersici* determined by large scale proteomics and cDNA assembly from 454 DNA sequencing. Plant Cell, Submitted

Von Caemmerer, S., Edwards, G., Koteyeva, N., and Cousins, A. Single cell C_4 photosynthesis in aquatic and terrestrial plants: a gas exchange perspective. Aquatic Bot, submitted.

Robert J. Spreitzer, University of Nebraska, Nebraska AES

Objective 2: Photosynthetic Capture and Photorespiratory Release of CO_2
Impact Statement:
A deeper understanding of the structure-function relationships of chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyzes the rate-limiting step of photosynthesis, will identify targets for engineering an increase in CO_2 fixation. The green alga *Chlamydomonas reinhardtii*, which is a well-developed model genetic organism for the study of photosynthesis, can be used as a host to facilitate the engineering of crop-plant Rubisco.

Accomplishments:
1. Identified an interface between chloroplast-encoded large subunits and nuclear-encoded small subunits that is responsible for the catalytic differences between algal and plant Rubisco enzymes.
2. In collaboration with M. E. Salvucci (Arizona ARS), demonstrated that small subunits influence Rubisco catalysis without altering the holoenzyme structural transition catalyzed by Rubisco activase.
3. Succeeded in creating functional Rubisco enzymes comprised of algal large subunits and plant small subunits, which determine algal pyrenoid formation.
4. Identified phylogenetic differences between algal and plant large subunits that can be engineered to express functional plant Rubisco holoenzyme in *Chlamydomonas*.

Plans for Coming Year:
- Define the minimal set of large-subunit substitutions required for expressing crop-plant Rubisco in *Chlamydomonas*.
- Use combinatorial site-directed saturation mutagenesis, coupled with genetic selection, to improve the catalytic efficiency of crop-plant Rubisco.
- Elucidate the role of large-subunit posttranslational modifications in the function of algal Rubisco.

Publications (2013):

Meyer, M.T., Genkov, T., Skepper, J.N., Jouhet, J., Mitchell, M.C., Spreitzer, R.J., and Griffiths, H. (2012). Rubisco small-subunit α-helices control pyrenoid formation in *Chlamydomonas*. Proc. Natl. Acad. Sci. USA **109:** 19474-19479.
Pinto, T.S., Malcata, F.X., Arrabaca, J.D., Silva, J.M., Spreitzer, R.J., and Esquível, M.G. (2013). Rubisco mutants of *Chlamydomonas reinhardtii* enhance photosynthetic hydrogen production. Appl. Microbiol. Biotechnol. **97:** 5635-5643.
Wachter, R.M., Salvucci, M.E., Carmo-Silva, A.E., Barta, C., Genkov, T., and Spreitzer, R.J. (2013). Activation of interspecies-hybrid Rubisco enzymes to assess different models for the Rubisco-Rubisco activase interaction. Photosynth. Res. **117:** 557-566.
Esquivel, M.G., Genkov, T., Nogueira, A.S., Salvucci, M.E., and Spreitzer, R.J. (2013). Substitutions at the opening of the Rubisco central solvent channel affect holoenzyme stability and CO_2/O_2 specificity but not activation by Rubisco activase. Photosynth. Res. **118:** 209-218.

Donald P. Weeks, University of Nebraska-Lincoln, Nebraska AES

Objective 2: Photosynthetic Capture and Photorespiratory Release of CO_2

Impact Statement:
We have successfully developed new and effective techniques for targeted gene knockout in higher plants based on the Clustered Regularly Interspersed Short Palindromic Repeats/CRISPR-associated 9/single guide RNA (CRISPR/Cas9/sgRNA) system recently shown to be effective in animal cells and a number of eukaryotic organisms. Attempts are underway to develop similar techniques for use in *Chlamydomonas reinhardtii* and other algae of interest in regard to studies of the CO_2-concentrating mechanism and algal biofuel production. The CRISPR/Cas9/sgRNA technology also shows strong promise for allowing the powerful manipulation of plant genetic information through gene replacement by homologous recombination. The ability to quickly and efficiently inactivate, modify or replace any gene of choice in higher plants opens significant new opportunities for fundamental studies of biochemical and molecular networks involved in plant growth and development - as well as practical applications of this new-found knowledge for modifying plants for production of higher quantities of more nutritious foods.

Accomplishments:
1. Demonstration that the CRISPR/Cas9/sgRNA system works accurately and efficiently in two model higher plants (Arabidopsis and tobacco) and two crop plants (rice and sorghum) for targeted gene disruption.
2. Development of a library of camelid antibodies against *Chlamydomonas* total-cell antigens and its use in isolating cell surface-specific V_HH antibodies.
3. Development of a set of fluorescent protein markers for use in tagging recombinant proteins expressed in *Chlamydomonas*.

Plans for Coming Year:
- Expand the use of CRISPR system technologies for gene modification/gene replacement in higher pants and algae.
- Apply the CRISPR/Cas9/sgRNA system to the development of higher plants producing foods with improved nutrition and safety.
- Apply the Chlamydomonas cell surface-specific, fluorescently tagged V_HH antibodies to the isolation and characterization of Chlamydomonas-related algae, determine the degree of their relatedness and characterize their lipid profiles.

Publications (2013):
Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., and Weeks, D.P. (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucl. Acids Res. (in press).
Jiang, W., Rosenberg, J.N., Wauchope, A.D., Tremblay, J.M., Shoemaker, C.B., Weeks, D.P., and Oyler, G.A. (2013). Generation of a phage display library of single-domain camelid V_HH antibodies directed against *Chlamydomonas reinhardtii* antigens and characterization of V_HHs binding cell surface antigens. Plant J. (in press).
Rasala, B., Barrera, D., Ng, J., Plucinak, T., Rosenberg, J., Weeks, D., Oyler, G., Peterson, T., Haerizadeh, F., Mayfield, S. (2013). Expanding the spectral palette of fluorescent proteins for the green microalga *Chlamydomonas reinhardtii*. Plant J. **74:** 545-556.

Patent: SINGLE CHAIN ANTIBODIES FOR PHOTOSYNTHETIC MICROORGANISMS AND METHODS OF USE. Application number: 20120277411, **Filed:** April 9, 2012, **Issued:** November 1, 2012. **Inventors:** George A. Oyler, Julian N. Rosenberg, Donald P. Weeks.

Christoph Benning, Michigan State University, AgBioResearch

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact Statement:
Insights into the regulation carbon partitioning of primary photosynthate, i.e. sugars, into high energy storage compounds, e.g. triacylglycerol, in plants and algae will guide the engineering of vegetable oil food crops and novel biofuel crops. A mechanistic understanding how algal cells enter a quiescent state following nutrient starvation and exit it following refeeding will provide novel means to address the problem cessation of growth during triacylglycerol accumulation in algae.

Accomplishments:
1. Introduced an algal diacylglycerol acyltransferase into Arabidopsis to enhance the triacylglycerol content in leaves and modify its fatty acid composition.
2. Identified a putative transcription factor in Chlamydomonas (CHT7) that controls the transition into and out of a nutrient-dependent quiescent state in Chlamydomonas.
3. Developed techniques to target proteins to the lipid droplet surface in Nannochloropsis.
4. Developed new mass spectrometry-based methods of triacylglycerol quantification and mutant screening in microalgae.
5. Identified phenotypes of Arabidopsis PGP1-like lipase mutants suggesting a role in abiotic stress responses.

Plans for Coming Year:
- Transfer the technology for production of oil in vegetative tissues to a representative monocot species such as Brachypodium.
- Develop a mechanistic understanding of how the CHT7 protein of Chlamydomonas affects entry/exit into/out of a nutrient induced quiescent state.
- Screen for novel lipid accumulation mutants in Nannochloropsis, which is a biotechnologically relevant microalgae with high levels or triacylglycerol.
- Determine the function of PGD1-like proteins in Arabidopsis.

Publications (2013):

Hemschemeier, A., Casero, D., Liu, B., Benning, C., Pellegrini, M., Happe, T., and Merchant, S.S. (2013). COPPER RESPONSE REGULATOR1-dependent and -independent responses of the *Chlamydomonas reinhardtii* transcriptome to dark anoxia. Plant Cell **25:** 3186-3211.
Liu, B., Vieler, A., Li, C., Jones, A.D., and Benning, C. (2013). Triacylglycerol profiling of microalgae *Chlamydomonas reinhardtii* and *Nannochloropsis oceanica*. BioRes.Tech. **146:** 310-316.
Ma, W., Kong, Q., Arondel, V., Kilaru, A., Bates, P.D., Thrower, N.A., Benning, C., and Ohlrogge, J.B. (2013). Wrinkled1, a ubiquitous regulator in oil accumulating tissues from Arabidopsis embryos to oil palm mesocarp. PloS One **8:** e68887.
Sanjaya, Miller, R., Durrett, T.P., Kosma, D.K., Lydic, T.A., Muthan, B., Koo, A.J., Bukhman, Y.V., Reid, G.E., Howe, G.A., Ohlrogge, J., and Benning, C. (2013). Altered lipid composition and enhanced nutritional value of Arabidopsis leaves following introduction of an algal diacylglycerol acyltransferase 2. Plant Cell **25:** 677-693.
Urzica, E.I., Vieler, A., Hong-Hermesdorf, A., Page, M.D., Casero, D., Gallaher, S.D., Kropat, J., Pellegrini, M., Benning, C., and Merchant, S.S. (2013). Remodeling of Membrane Lipids in Iron-starved *Chlamydomonas*. J. Biol. Chem. **288:** 30246-30258.

John C. Cushman, University of Nevada, Nevada AES

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact statement:
As an alternative to transgenic approaches used to manipulate microalgae strains to enhance lipid or starch production, reiterative, transgressive selection strategies based upon flow cytometry, fluorescence-activated cell sorting, and buoyant density centrifugation have been used successfully to select and isolate algal strains with altered feedstock characteristics such as greater starch or lipid accumulation.

Accomplishments:
Strain selection strategies for improvement of algal biofuel feedstocks
Sustainable production of renewable fuels is needed as reserves of fossil petroleum become depleted and its damaging impact on the environment becomes more apparent. In recent decades, microalgae have gained a great deal of interest as feedstocks for biofuel production because they are able to produce large amounts of lipids and starch, and can serve as a non-seasonal renewable energy crop that can be grown with fresh, waste, brackish, or salt water on marginal lands currently considered unusable for traditional agricultural applications. Although a number of genetic engineering approaches have been developed to manipulate the lipid or starch production of selected algal strains, environmental release of genetically modified strains carries with it concerns. Reiterative, transgressive selection strategies have been developed and provide an alternative, non-transgenic approach for manipulating microalgae strains to enhance lipid or starch production. Various selection methods, based upon flow cytometry, fluorescence-activated cell sorting, and buoyant density centrifugation, have been used successfully to select and isolate algal strains with altered feedstock characteristics such as greater starch or lipid accumulation (Hathwaik and Cushman, 2013).

Objective 4: Developmental and Environmental Limitations to Photosynthesis
Impact statement:
Crassulacean acid metabolism (CAM) is an alternative mode of photosynthesis that improves water-use efficiency. Plans are underway to describe in detail the biochemical and regulatory requirements of CAM using comparative genomics approaches among taxonomically diverse CAM species. Such information will inform synthetic biology strategies to move CAM into C_3 crops thereby improving their water-use efficiency.

Accomplishments:
Engineering crassulacean acid metabolism (CAM) into C_3 crops to improve water-use efficiency
Emerging technologies of synthetic biology offer one approach to improve plant water-use efficiency (WUE) by introducing crassulacean acid metabolism (CAM) into C_3 crops. Efforts are underway to achieve comprehensive systems-level understanding of the enzymatic and regulatory pathways underpinning this temporal CO_2 pump. As CAM arose through multiple independent evolutionary origins, comparative transcriptomics and genomics of taxonomically diverse CAM species are being used to define the genetic 'parts list' required to operate the core CAM functional modules of nocturnal carboxylation, daytime decarboxylation, and inverse stomatal regulation. Bioengineered CAM offers the potential to sustain plant productivity for food, fibre, and biofuel

production in the face of hotter and drier climates (Borland et al., 2013; DePaoli et al., 2013).

Plans for Coming Year:

- Complete functional genomics analyses in wild type common ice plant *(Mesembryanthemum crystallinum* L.), a facultative CAM species, including the completion of genome sequencing. Analysis of a thick-leaf mutant of ice plant will also be included.
- Complete functional genomics analyses in prickly pear cactus *(Opuntia ficus-indica*), an obligate CAM species, under water deficit stress and during development, including draft genome sequencing. Transcriptome and genome sequence information about Rubisco activase from *Opuntia* will be used in collaboration with Mike Salvucci (Arizona ARS) to examine the thermotolerance of this enzyme from this cactus species.
- Design carboxylation, decarboxylation, and stomatal control modules to move CAM into C_3 model (*Arabidopsis*) and biofuel crops (*Populus*). Explore productive strategies to engineer increases in leaf tissue succulence.
- Report on the use of reiterative (and transgressive) selection methods based upon flow cytometry, fluorescence-activated cell sorting, and buoyant density centrifugation to select and isolate *Dunaliella salina* cell lines with either increased lipid or starch accumulation.

Publications (2013):

Borland, A.M., Hartwell, J., Weston, D., Schlauch, K.A., Tschaplinski, T.J., Tuskan, G.A., Yang, X., and Cushman, J.C. (2013). Engineering Crassulacean acid metabolism to improve water-use efficiency. Trends Plant Sci. Submitted.

DePaoli, H.C., Borland, A.M., Tuskan, G.A., Cushman, J.C., and Yang, X. (2013). Synthetic biology as it relates to CAM photosynthesis: Challenges and opportunities. J. Exp. Bot. Submitted.

Hathwaik, L.T., and Cushman, J.C. (2013). Strain selection strategies for improvement of algal biofuel feedstocks. In: Biofuels and Bioenergy. Eds: John Bryant, Clive Butler, and John Love. Wiley-Blackwell, UK. Submitted.

Samburova, V., Lemos, M.S., Hiibel, S.R., Hoekman, S.K., Cushman, J.C., and Zielinska, B. (2013). Analysis of triacylglycerols and free fatty acids in algae using ultra-high-pressure liquid chromatography mass spectrometry. J. Am. Oil Chem. Soc. **90:** 53-64.

Wone, B.W., Donovan, E.R., Cushman, J.C., Hayes, J.P. (2013). Metabolic rates associated with membrane fatty acids in mice selected for increased maximal metabolic rate. Comp. Biochem. Physiol. Mol. Integr. Physiol. **165:** 70-78.

Yobi, A., Wone, B.W.M., Guo, L., Alexander, D.C., Ryals, J.A., Oliver, M., and Cushman J.C. (2013). Metabolomic profiling in *Selaginella lepidophylla* provides new insights in the mechanistic basis of desiccation tolerance. Molec. Plant. **6:** 369-385.

Yobi, A., Schlauch, K.A., Perryman, B., Oliver, M.J., and Cushman, J.C. (2013). Biomass production, nutritional, and mineral content of desiccation-sensitive and desiccation-tolerant species of *Sporobolus* under multiple irrigation regimes. J. Agron. Crop Sci. **199:** 309-320.

Glenda E. Gillaspy, Virginia Tech, Virginia AES

Objective 3. Mechanisms regulating photosynthate partitioning
Impact Statement:
Glenda Gillaspy (VT) has investigated the mechanisms that regulate sugar sensing and photosynthate partitioning into biosynthetic pathways, related to Objective 3 of the project. The sucrose non-fermenting related kinases (SnRKs) are major energy sensors in eukaryotes that impact global transcription programs, stress signaling and lifespan. Work is on-going to delineate the regulation of expression of different SnRK1 genes and protein isoforms, and to delineate which isoforms elevate plant biomass when expressed in transgenic plants. A separate approach that addresses sugar sensing focuses on inositol pyrophosphate signaling molecules, which are important players in eukaryotic energy and metabolic regulation. Characterization of plant genes involved in inositol pyrophosphate synthesis was reported.

Accomplishments:
1. Characterized the spatial expression profiles and subcellular location of two Arabidopsis SnRK1 genes/proteins.
2. Constructed transgenic plants containing overexpression of the various SnRK1 protein isoforms to delineate sequences necessary for biomass changes in transgenic plants.
3. Identified that different plants synthesize InsP7 and the relative accumulation of this molecule in both vegetative and embryonic tissues, and identified kinase genes in Arabidopsis capable of driving InsP7 synthesis in yeast mutants.

Plans for Coming Year:
- Determine the amino acid sequences necessary for SnRK1-induced alterations in plant biomass.
- Characterize the impact of SnRK1 expression in transgenic cotton.
- Examine the regulation of inositol pyrophosphates in Arabidopsis and cotton.

Publications (2013):
Golani, Y., Kaye, Y., Hassidim, M., Ercetin, M., Gillaspy, G. and Levine, A. (2013). Inositol polyphosphate phosphatidylinositol 5-phosphatase 9 (At5PTase9) controls plant salt tolerance by regulating endocytosis. Mol Plant. (in press).
Gillaspy, G. (2013). The role of phosphoinositides and inositol phosphates in plant cell signaling. Capelluto, D. (editor), In Lipid-Mediated Protein Signaling, Springer Science & Business Media, LLC (in press).
Donahue, J., Ercetin, M., Gillaspy, G. (2013). Assaying inositol and phosphoinositide phosphatase enzymes. Heilmann I and Munnik T (eds.), Methods Mol. Biol. **1009:**175-85.
Torrens-Spence, M.P., Liu, P., Ding, H., Harich, K., Gillaspy, G., Li, J. (2013). Biochemical evaluation of the decarboxylation and decarboxylation-deamination activities of plant aromatic amino acid decarboxylases. J. Biol. Chem. **288:** 2376-87.

Mike Giroux, Montana State University, Montana AES

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact Statement:
The long term goal of the proposed research is to increase cereal agronomic yield. This goal may be achieved through further understanding of how source and sink strength influence plant yield. Here we focus on starch, as it is an important metabolite in both sink and source strength. Starch biosynthesis in the endosperm drives sink strength, whereas leaf starch is an important contributor of source strength and thus plant growth as excess photosynthate is stored as starch during the light period and remobilized during the dark period. This project is focused on understanding the importance of both leaf and seed starch biosynthesis in terms of metabolic, transcriptomic, and developmental factors currently limiting plant productivity in order to improve plant production.

Accomplishments:
1. Created transgenic rice with overexpression of leaf and/or seed starch biosynthetic rates.
2. Developed methods for measuring rice yield throughout plant development in growth chambers.

Plans for Coming Year:
- Conduct yield trials of transgenic rice with increases in seed and/or leaf starch levels.
- Determine impact of increased starch on rice growth throughout development.

Publications (2013):

Schlosser, A.J., Martin, J.M., Hannah, L.C., and Giroux, M.J. (2012). The maize leaf starch mutation agps-m1 has diminished field growth and productivity. Crop Sci. **52:** 700-706.

Steven C. Huber, University of Illinois, Illinois AES/ARS

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact Statement:
New insights about the regulatory mechanisms controlling protein kinase activity, such as the role of flanking domains and interactions with other proteins such as calmodulin, are modifying paradigms and may provide targets for manipulation in future studies to regulate growth, stomatal aperture, hormone signaling, and immune/stress signaling in crop plants to favor productivity.

Accomplishments:
1. At least some CDPKs are dual-specificity kinases, capable of phosphorylation on serine, threonine, and tyrosine residues. In addition, while the CDPKs do not require calmodulin for activation by Ca^{2+}, nonetheless the CDPKs do bind calmodulin in a Ca^{2+}-dependent manner with functional impact on CDPK activities.
2. Determined that tyrosine autophosphorylation sites on CDPKs are fully accessible to protein phosphatases, despite structural modeling studies suggesting that they are buried. This implies conformational breathing and flexing.
3. Established that the carboxy-terminal domain of the receptor kinase, BAK1, is an important regulator of kinase activity and signaling in vivo that impacts plant growth.
4. Canopy position affects the concentration of iron in soybean seeds (grown in Urbana, IL, or Africa). Seed at the bottom of the canopy contain 20 – 30% higher [Fe] than seeds at the top of the canopy and flour, milk, okara produced from the seeds reflect those differences. Genotype differences in absolute [Fe] also exist. These results have implications for use of soybeans as human food in developing countries where iron in the diet is chronically insufficient.

Plans for Coming Year:
- Determine functional impact of calmodulin binding to protein kinases in vivo.
- Use TALENs to knockout the large subunit of soybean Rubisco activase (Spalding; Salvucci).
- Attempt TALEN-mediated directed mutagenesis of regulatory cysteine residues in Arabidopsis activase (Spalding; Salvucci).
- Characterize the phosphorylation of Arabidopsis Activase and its functional significance (Spalding; Salvucci).

Publications (2013):

Bajwa, V.S., Wang, X., Blackburn, R.K., Goshe, M.B., Mitra, S.K., Williams, E.L., Bishop, G.J., Krasnyanski, S., Allen, G., Huber, S.C., and Clouse, S.D. (2013). Identification and functional analysis of tomato BRI1 and BAK1 receptor kinase phosphorylation sites. Plant Physiol. **163:** 30-42.
Macho, A., Schwessinger, B., Ntoukakis, V., Brutus, A., Segonzac, C., Roy, S., Kadota, Y., Malinovsky, F.G., Monaghan, J., Oh, M.H., Huber, S.C., He, S.Y., and Zipfel, C. (2013). A bacterial tyrosine phosphatase targets activation of plant pattern recognition receptors. Nature (submitted).

JC Jang, Ohio State University, Ohio AES

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:
Although numerous reports have shown that plant Tandem CCCH Zinc Finger proteins (TZFs) are powerful regulators of sugar and hormone-mediated plant growth and stress response, the underlying molecular mechanisms are obscure. Our work has provided convincing evidence suggesting that plant TZF proteins modulate gene expression by targeting specific RNA elements and affecting post-transcriptional regulation. Specifically, we have demonstrated that 1) TZF expression is induced by sugar and hormone, 2) TZFs can bind RNA with specificity, 3) TZFs are localized in P-bodies and stress granules, two novel structures involved in RNA regulation, 4) TZFs can modulate sugar-, ABA-, and GA-responsive genes.

Accomplishments:
1. Demonstrate that Arabidopsis TZF1 binds unorthodox RNA elements via a unique RNA binding domain.
2. Determine the roles of seed-specific AtTZF4, AtTZF5, and AtTZF6 in phytochrome/hormone mediated seed germination and GA/ABA-mediated plant growth response.
3. Identification of genetic components involved in AtTZF1-dependent regulatory pathways.

Plans for Coming Year:
- Genome-wide identification of AtTZF1 mRNA targets.
- Molecular characterization of AtTZF1-RNA interaction.
- Determine the regulatory impacts of AtTZF1 on mRNA fate.
- Characterization of suppressors of AtTZF1 mutants.

Publications (2013):

Bogamuwa, S., and Jang, J.-C. (2013). The Arabidopsis tandem CCCH zinc finger proteins AtTZF4, 5, and 6 are involved in light-, ABA- and GA-mediated regulation of seed germination. Plant Cell Environment **36:** 1507-1519.
Qu, J., Kang, S.G., Wang, W., Musier-Forsyth, K., and Jang, J. C. (2013). RNA binding activity of recombinant *Arabidopsis thaliana* tandem zinc finger 1 (AtTZF1) is mediated via plant-unique arginine-rich domain and TZF domain. Plant J. (submitted).

Karen E. Koch, University of Florida, Florida AES

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact Statements:

- The strigolactone-deficient maize mutant (*ccd8*) shows the vital role of this hormone in C-partitioning to the root system of maize (primarily prop roots), and to ears with altered architecture.
- The *ccd8* mutant also provides a potentially invaluable source of maize resistance to *Striga* (a deadly parasitic weed that reduces yields to near zero in much of Africa).
- New research materials and tools for using them have been made available through the UniformMu Maize Resource and the recently-developed Mu-seq protocols (The latter facilitate analysis of genotype-phenotype relationships in these mutants).

Accomplishments:

1. We identified and characterized maize mutants dysfunctional for strigolactone production, and its effect on altered C-partitioning, architecture, ear morphology, and possible *Striga* resistance.
2. We developed Mu-seq protocols for identifying Mu-insertions and analyzing genotype-phenotype associations in transposon-based mutant resources.
3. We released 576 new mutant lines carrying >10,000 mapped insertions in the Uniform Mu Maize Resource, which now includes insertions in >40 of maize genes (available via MaizeGDB.org and the Maize Genetics Cooperative Stock Center).

Plans for Coming Year:

- We will further pursue effects of strigolactone on photosynthate partitioning and if possible, the impact of its deficiency on *Striga* resistance of maize.
- We will continue to characterize maize mutants with potential to alter photosynthate partitioning and sugar sensing. Emphasis will be directed towards kernel and ear features, but other aspects of C4-panicoid biology will also be addressed.

Publications (2013):

Bihmidine, S., Hunter, C.T., Johns, C.E., Koch, K.E., and Braun, D. (2013). Regulation of assimilate import into sink organs: Update on molecular drivers of sink strength. Frontiers in Plant Physiology **4:**177 (15 pgs).
McCarty, D.R., Latshaw, S., Wu, S., Suzuki, M., Hunter, C.T., Avigne, W.T., and Koch, K.E. (2013). Mu-seq: sequence-based mapping and identification of transposon induced mutations. PloS ONE **8:** e77172
Djidonou, D., Zhao, X., Simonne, E.H., Koch, K.E., and Erickson, J.E. (2013). Yield, water-, and nitrogen-use efficiency in field-grown, grafted tomatoes. HortScience **48:** 485-492.
Wang, J., Nayak,S., Koch, K., and Ming, R. (2013). Carbon partitioning in sugarcane (*Saccharum* species). Frontiers in Plant Science **4:** 201 (6 pgs).

Other:
Named “Fellow,” ASPB and AAAS. Elected National Secretary for ASPB.

Thomas D. Sharkey, Michigan State University AgBioResearch

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Impact Statement:
The products of photosynthesis are partitioned, first between sucrose and starch and then between growth of new leaves, roots, flowers and seeds. Partitioning between starch and sucrose is highly regulated and disruption of this regulation can lead to starvation signals or accumulation of starch instead of new growth. The relative growth rate of a plant is extremely sensitive to partitioning because partitioning to new leaves increases photosynthetic capacity and so is exponential while partitioning to roots, stems, flowers, or seeds allows linear growth in biomass and no growth in leaf area.

Accomplishments:
1. Several transgenic lines of Arabidopsis were made that have an extra glucose 6-phosphate transporter that normally is not present in leaves (only in roots).
2. A plant growth model that examines the relationship between growth in leaf area versus biomass growth was developed.
3. Measurements were made with genotypes that alter partitioning to leaf area growth and the effect on relative growth rates were determined.

Plans for Coming Year:
- Analyze the transgenic plants for starch/sucrose partitioning regulation.
- Increase the complexity of the model so that effects of small changes in partitioning to leaf area, leaf thickness, starch, root growth, and stem growth can be modeled to determine optimal growth strategies.
- Increase the range of genotypes subjected to growth analysis to study how partitioning affects growth rate.

Publications (2013):
No publications in 2013

Robert Aiken and Vara Prasad, Kansas State University, Kansas AES

Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:

We have improved our understanding of genetic variability, mechanisms, and field responses associated with improved tolerance of drought (water use efficiency and water availability) and supra-optimal temperature. In sorghum, water use efficiency (WUE) was more strongly associated with biomass than water use. Stay-green trait in sorghum was not associated with sensitivity of transpiration to either soil drying or vapor pressure deficits. High day or nighttime temperatures increased respiration rates and decreased photosynthesis, pollen germination and seed-set percentage in soybean. High temperature stress caused biochemical, anatomical and morphological changes in soybean pollen grains leading to decreased pollen germination and seed-set. Water deficits reduced wheat productivity responses to an increment of water use and impaired multiple components of grain formation.

Accomplishments:

1. Eight sorghum genotypes were evaluated for biomass production, WUE, and radiation use efficiency (RUE) and to test whether the differences in WUE among sorghum genotypes were associated with increased biomass production or decreased water use under field conditions. It was observed that WUE was more strongly correlated with biomass production than to water use. These results imply that it is possible to improve WUE without compromising biomass production (Narayanan et al., 2013).
2. Persistence of green leaves during seed-filling, referred to as a stay-green trait, has been investigated in sorghum as an approach to increase yields under water-limited conditions. We tested two potential mechanisms of water conservation using a set of 12 contrasting sorghum genotypes: first, an earlier decrease in transpiration with soil drying so that rate of soil water loss is decreased earlier in the soil drying cycle; and second, a limitation on transpiration rate at high vapor pressure deficits (VPD), so that soil water is conserved on days when midday VPD is high). Expression of stay-green traits was measured under field conditions. Results indicated that there was no evidence that stay-green trait was closely linked with either mechanism of water conservation (Choudhary et al., 2013a).
3. The impact of leaf hydraulic conductance on water use characteristics was explored by comparing two contrasting sorghum genotypes. Genotype SC15 had a much lower leaf conductance than genotype SC1205. Results demonstrated that low hydraulic conductance in SC15 was associated with conservative water use by restricting transpiration at higher soil water content during soil drying and under high VPD (above 2.1 kPa). Genotype SC1205 did not show such response. Tests with inhibitors indicate that these differences may be linked to differences between their aquaporin populations (Choudhary et al., 2013b).
4. Impact of high day-or nighttime temperatures was quantified on leaf photosynthesis, respiration, pollen germination, pod-set percent and seed weights. Plants were exposed to high daytime (39/20°C), high nighttime (30/23, 30/26 or 30/29°C), or optimum (30/20°C) temperature for 10 days at flowering. High day-or nighttime temperature increased leaf respiration and decreased photochemical quenching, electron transport rate and leaf photosynthesis, and pollen germination. Overall, high temperatures during flowering decreased leaf photosynthesis and pollen germination, leading to decreased pod-set percentage and seed weight (Djanaguiraman et al., 2013a). High temperature stress resulted in deformed pollen grains with thicker exine walls and disintegrated tapetum layer leading to decreased pollen germination and pod-set percentage (Djanaguiraman et al., 2013b).
5. Long-term crop sequence studies demonstrated that water deficits, associated with annual cropping, reduced water productivity of wheat, relative to wheat grown after an 11-month

fallow period. Above-ground biomass and grain productivity response to an increment of water use were reduced 18 and 31%, for wheat in annual cropping systems, relative to wheat grown after fallow. Under severe water deficit conditions, sink-limitations to grain formation was indicated by reduced harvest index (grain fraction of above-ground biomass; Aiken et al., 2013).

Plans for Coming Year:

- Evaluate impact of high temperature stress on reproductive processes and yield formation in grain crops.
- Quantify genetic variability for traits associated with high temperature (photosynthesis and reproductive success) and drought tolerance (rooting depth and fine roots) in germplasm collections of grain crops.
- Understand mechanisms associated with tolerance for high temperature and drought tolerance.
- Develop and apply novel phenotypic methods to screen germplasm for tolerance to high temperature and drought stress in controlled environments in laboratory and field conditions.

Publications (2013):

Aiken, R.M., O'Brien, D.M., Olson, B.L. and Murray, L. 2013. Replacing fallow with continuous cropping reduces crop water productivity of semiarid wheat. Agron. J. **105:**199-207.

Choudhary, S., Mutava, R.N., Shekoofa, A., Sinclair, T.R., and Prasad, P.V.V. 2013a. Is the stay-green trait in sorghum a result of transpiration sensitivity to either soil drying or vapor pressure deficit?. Crop Science **53:** 2129-2134.

Choudhary, S., Sinclair, T.R., and Prasad, P.V.V. 2013b. Hydraulic conductance of intact plants of two contrasting sorghum lines SC15 and SC1205. Functional Plant Biology **40:** 730-738.

Craufurd, P.Q., Vadez, V., Jagadish, S.V.K., Prasad, P.V.V., and Zaman-Allah, M. 2013. Crop science experiments designed to inform crop modeling. Agricultural and Forest Meteorology 170: 8-18.

Djanaguiraman, M., Prasad, P.V.V., and Schapaugh, W.T. 2013a. High day and night temperature alters leaf assimilation, reproductive success and phosphatidic acid of pollen grain in soybean (*Glycine max* L. Merr.). Crop Science **53:** 1594-1604.

Djanaguiraman, M., Prasad, P.V.V., Boyle, D.L., and Schapaugh, W.T. 2013b. Soybean pollen anatomy, viability and pod set under high temperature stress. Journal of Agronomy Crop Science **199:** 171-177.

McMaster, G.S., Ascough II J.C., Edmunds, D.A., Neilsen, D.C., and Prasad, P.V.V. 2013. Simulating crop phenological responses to water stress using the phenologyMMS software program. Applied Engineering in Agriculture **29:** 233-249.

Narayanan, S., Aiken, R.A., Xin, Z., Prasad, P.V.V., and Yu, J. 2013. Water use efficiencies in sorghum. Agronomy Journal **105:** 649-656.

Paul, G., Gowda, P.H., Prasad, P.V.V., Howell, T.A., Staggenborg, S.A., and Neale, C.M.U. 2013. Lysimetric evaluation of SEBAL using high resolution airborne imagery from BEAREX08. Advances in Water Resources **59:** 157-168.

Rao, S.S., Patil, J.V., Prasad, P.V.V., Reddy, D.C.S., Mishra, J.C., Umakanth, A.V., Reddy, B.V.S., and Kumar, A.A. 2013. Sweet sorghum planting effects on stalk yield and sugar quality in semi-arid tropical environment. Agronomy Journal **105:** 1458-1465.

Singh, R.P., Reddy, K.R., and Prasad, P.V.V. 2013. Impact of changing climate and climate variability on seed production and seed industry. Advances in Agronomy **118:** 49-110.

Fred E. Below and Laura F. Gentry, University of Illinois, Illinois AES

Objective 4: Developmental and Environmental Limitations to Photosynthesis
Impact Statement:
Understanding the interaction of environment, genetic, and management factors in altering light interception, photosynthesis and corn productivity is needed to feed a growing world population. Work in 2013 helped to quantify the roles of genetic and transgenic maize improvement on plant mineral nutrient use; the need for higher plant populations to optimize biomass production and corn yield; and the ability to use tropical maize hybrids as a biomass and sugar source for bioethanol production.

Accomplishments:
1. Assessing how genetic improvement in maize yield impacts nitrogen use parameters
2. Quantifying effects of input intensification on above- and belowground corn plant biomass production
3. Identification of factors controlling the yield penalty in continuous corn production
4. Understanding the impact of transgenic corn rootworm protection on accumulation, partitioning, and use of mineral nutrients
5. Interactions of plant density and fertilizer application on corn yield

Plans for Coming Year:
- Estimating loss of crop yield and biomass productivity resulting from inadequate/untimely precipitation or nutrient availability in corn production systems
- Assessing tropical maize as an emerging animal feed and bioenergy crop
- Integrating cover crops into tropical maize production systems
- Understanding interactions of row spacing and plant density on yield of maize

Publications (2013):
Gentry, L.F., **Ruffo**, **M.L., and Below, F.E.** (2013). Identifying factors controlling the continuous corn yield penalty. Agron. J. **105:** 295-303.
Haegele, J.W., and Below, F.E. (2013). Transgenic corn rootworm protection increases grain yield and nitrogen use of maize. Crop Sci. **53:** 585-594.
Haegele, J.W., Cook, K.A., Nichols, D.M., and Below, F.E. (2013). Changes in nitrogen use traits associated with genetic improvement for grain yield of maize hybrids released in different decades. Crop Sci. **53:** 1256-1268.
Bender, R.R., Haegele, J.W., Ruffo, M.L., and Below, F.E. (2013). Nutrient uptake, partitioning, and remobilization in modern, transgenic insect-protected maize hybrids. Agron. J. **105:** 161-170.
Bender, R.R., Haegele, J.W., Ruffo, M.L., and Below, F.E. (2013). Transgenic corn rootworm protection enhances uptake and post-flowering mineral nutrient accumulation. Agron. J. **105:** 1626-1634.
Chen, M.H., Kaur, P., Dien, B., Below, F.E., Vincent, M.L, and Singh. V. (2013). Use of tropical maize for bioethanol production. World J. Microbiol. Biotechnol. **29:** 1509-1515.
Haegele, J.W., Becker, R.J., Henninger, A.S., and Below, F.E. (2013). Row arrangement, phosphorus fertility, and hybrid contributions to managing increased plant density of maize. Agron. J. (in press).

Jiaxu Li, Mississippi State University, Mississippi AES

Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:
Abscisic acid is an important hormone in regulating plant responses to abiotic stresses such as drought and high salinity. The abscisic acid function is mediated by abscisic acid-activated protein kinases and kinase-catalyzed protein phosphorylation. Therefore identification of the target proteins of abscisic acid-activated protein kinases will reveal new components in abscisic acid signaling pathways.

Accomplishments:
1. Phosphoproteins have been isolated from abscisic acid-activated protein kinase knockout mutants (*snrk2.2* and *snrk2.3*) and wild-type Arabidopsis plants.
2. Ten differential phosphoproteins in response to abscisic acid treatments have been identified.

Plans for Coming Year:
We will do functional analysis of some identified phosphoproteins to determine their roles in abscisic acid signaling.

Publications (2013):
Manuscript in preparation

NC-1200 Multi-State Research Project
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