NC1200 – Regulation of Photosynthetic Processes
Annual Meeting and Reports

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NC1200 – Regulation of Photosynthetic Processes Program - 2015 Annual Meeting

Location: Sheraton Westport Plaza
900 Westport Plaza, St. Louis, MO 63146
Phone: 314-878-1500

Meeting room: Plaza 2/3

Friday, Nov. 6, 2015

19:00 Meet in hotel lobby, find restaurant for dinner

Saturday, Nov. 7, 2015

Breakfast on your own.

Beverages provided throughout the day: coffee, selection of teas, soft drinks and bottled water

8:30 Tasios Melis – University of California, Berkley

9:00 Michael Giroux – Montana State University

9:30 Jeffrey Harper – University of Nevada, Reno

10:00 Break

10:30 Helmut Kirchhoff – Washington State University

11:00 Rob Aiken - Kansas State University

11:30 Christoph Benning – Michigan State University

12:00 Lunch – Deli Lunch Buffet provided in meeting room

13:00 Doug Allen – USDA-ARS/Danforth Center, St Louis

13:30 Vara Prasad – Kansas State University

14:00 Glenda Gillaspy – Virginia Tech

14:30 Krishna Jagadish – Kansas State University (Guest)

15:00 Break

15:30 Felix Fritschi – University of Missouri

16:00 Business Meeting

18:30 Meet in hotel lobby, find restaurant for dinner

Helmut Kirchhoff, Institute of Biological Chemistry, Washington State University

Objective 1: Understanding architectural dynamics in plant thylakoid membranes

Impact Statement: The structure of stacked grana thylakoid membranes in plants is not static but highly dynamic, changing its shape in response to environmental cues. These architectural dynamics of the grana thylakoids are involved in regulating and maintaining photosynthetic performance. Therefore, the knowledge how thylakoid membranes change their shape and identification of functional consequences of structural alterations is required for an in-depth understanding of photosynthetic energy transformation.

Accomplishments:

1. We have found that light causes swelling of the thylakoid lumen. This is a paradigm shift since previous studied postulated light-induced shrinkage of the lumen. The swelling of the lumen activates plastocyanin-dependent electron transport by facilitating its diffusion through the crowded luminal space.

2. We identified that under high light, only the margins of the grana stacks swell but the grana core remains tightly stacked. This preferential swelling of the grana periphery is important for protein repair because it allows better access of proteases to damaged PSII. These proteases catalyze degradation of damaged D1, i.e. a critical step in the PSII repair.

3. The proteins in grana (PSII, LHCII) can sometimes rearrange from disordered to highly ordered, semicrystalline structures. The role of this supramolecular reorganization is not known. By using a model plant that constitutively has a high abundance of semiscrystalline protein arrays, we found that protein ordering facilitates diffusion of small lipophilic metabolites (plastoquinone, xanthophylls) but impairs mobility of larger protein supercomplexes. Thus, dynamic reorganization of protein order in grana fine-tunes diffusion dependent electron transport and photoprotection or protein repair.

Plans for Coming Year:

- Determine factors that control dynamic swelling/shrinkage of the thylakoid lumen. This includes studies on channel mutants, kinase/phosphatase mutants, and the newly discovered CURT protein family (required for bending grana margins).
- Measuring detailed light dependence of swelling and shrinkage and determine the kinetics of these processes
- Determine the role of dynamics of the lipid matrix composition on supramolecular protein organization in grana.

Publications (2014):

Puthiyaveetil, S., Tsabari, O., Lowry, T., Lenhert, S., Lewis, R.R., Reich, Z., Kirchhoff, H. (2014). Compartmentalization of the Protein Repair Machinery in Photosynthetic Membranes. Proc. Natl. Acad. Sci. USA **111**, 15839-15844.

Puthiyaveetil, S., Woodiwiss, T., Knoerdel, R., Zia, A., Wood, M., Hoehner, R., Kirchhoff, H. (2014). Significance of the Photosystem II Core Phosphatase PBCP for Plant Viability and Protein Repair in Thylakoid Membranes. Plant Cell Physiology **55**, 1245-1254.

Wensel, P., Helms, G., Hiscox, B., Davis, W.C., Kirchhoff, H., Bule, M., Yu, L., Chen, S. (2014). Isolation, characterization, and validation of oleaginous, multi-trophic, and haloalkaline-tolerant microalgae for two-stage cultivation. Algal Research **4**, 2-11.

Kirchhoff H. (2014). Structural changes of the thylakoid membrane network induced by high-light stress in plant chloroplasts. Philosophical Transactions B **369**, 20130225.

Kirchhoff H. (2014). Dynamic architecture of plant photosynthetic membranes. In: Advances in Plant Biology: Plastid Biology (Editors: F.-A. Wollman and S. Theg), Springer Press, 129-154.

Kirchhoff H. (2014). The mechanisms of repair. Research Media (UK), Nov 2014, 17-19

Kirchhoff H. (2014). Diffusion of molecules and macromolecules in thylakoid membranes. Special issue on Dynamics and Ultrastructure of Bioenergetic Membranes and their Components. Biochimica et Biophysica Acta-Bioenergetics **1837**, 495-502.

Christoph Benning, Michigan State University, AgBioResearch

Impact Statement:
Insights into the regulation of 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 conundrum of cessation of growth during maximal accumulation of triacylglycerols in algae. Lipid remodeling under abiotic stress conditions is investigated as an important contributor to productivity in crop plants and algae.

Accomplishments:
Under Objective 1:
1. Analysis of a Chlamydomonas mutant deficient in the TGD2 protein was completed. It was determined that Chlamydomonas uses ER-derived precursors for thylakoid lipid biosynthesis.
2. The Brachypodium TGD1 protein was found to only partially complement the Arabidopsis *tgd1* mutant. Lipid trafficking was partially restored but activation of SFR2 was not reversed. Partial repression of TGD1 in Brachypodium lines does lead to mild lipid trafficking phenotypes but does not activate SFR2 as observed for Arabidopsis.

Under Objective 3:
3. Phenotypic analysis of the CHT7 mutant cells of Chlamydomonas has shown misregulation of cell cycle genes during N-deprivation. This is likely causal to other newly observed morphological cell phenotypes observed during N deprivation and N-refeeding.
4. The mechanism of lipid droplet formation in Chlamydomonas under different growth conditions has been investigated. A role for the major lipid droplet protein of Chlamydomonas in the targeting of proteins to the lipid droplet was identified.
5. The major enzymes involved in triacylglycerol formation in Nannochloropsis have been identified and obtained in active recombinant form. A forward genetic mutant screen for lipid mutants of Nannochloropsis was conducted and led to the identification of promising mutants. Mapping of the affected genes is under way.
6. The ectopic expression of WRI1 in Brachypodium led to increased lipid droplet formation in leaf blades but also induced localized cell death. It was determined that this phenotype was due to accumulation of free fatty acids which are toxic to cells. New construct were tested expressing WRI1 and other genes from algae under the control of stem-specific promoters. New, more stable variants of WRI1 were identified that led to higher accumulation of triacylglycerol in overexpressing tissues.

Under Objective 4:
7. Recombinant SFR2 protein of tomato has been obtained in its active form. RNAi repression of SFR2 in tomato led to increased salt and drought sensitivity.
8. Three PGDL proteins of Arabidopsis have been produced in their recombinant form and their lipase activity has been characterized. The proteins were found to be

associated with different chloroplast membranes. Overexpression of these proteins led to changes in thylakoid membrane lipid profiles.

9. The low triacylglycerol l mutant of Chlamydomonas, *pdgl1¸* was characterized under different growth conditions during incubation in photobioreactors. The mutant showed altered chlorophyll fluorescence and composition of the photosynthetic apparatus as well as increased light sensitivity.

Plans for Coming Year:

1. A focus will be on the refinement of in vitro transport assays to determine the lipid substrates for TGD proteins. The analysis of the role of SFR2 proteins in tomato will be completed and published. The analysis of recombinant PGDL proteins will be completed and published. The focus will shift to gain a better understanding of the function of the PGDL proteins in vivo through the detailed analysis of the respective mutants.
2. A major effort will be on the identification of direct target genes of the CHT7 complex in Chlamydomonas. A more in-depth analysis of the *cht7* mutant phenotype will be published. The physiological analysis of the Chlamydomonas *pgd1* mutant will be completed and published. The analysis of acyltransferases involved in triacylglycerol formation in Nannochloropsis will be completed and published. Their inactivation using CRISPR technology will developed. Selected lipid mutants of Nannochloropsis will be characterized to identify the mutated gene.
3. The analysis of Brachypodium lines partially deficient in the TGD1 protein will be completed. New transgenic Brachypodium lines expressing different genes potentially enhancing vegetative oil accumulation will be generated and characterized.

Publications (2014/2015 since last report):

1. Panchy, N., Wu G., Newton, L., Tsai, C.-H., Chen, J., Benning, C. Farre, EM, Shiu, S.-H. 2014. Prevalence, evolution and *cis*-regulation of diel transcription in *Chlamydomonas reinhardtii.* G3, doi:10.1534/g3.114.015032
2. Tsai, CH, Zienkiewicz, K, Amstutz, CL, Brink, BG, Warakanont, J, Roston, R, Benning C. 2015. Dynamics of protein and polar lipid recruitment during lipid droplet assembly in *Chlamydomonas reinhardtii*. Plant J. 83:650-660
3. Ma, W., Kong, Q., Grix, M., Mantyla, J. J., Yang, Y., Benning, C., Ohlrogge, JB. 2015. Deletion of a C-terminal intrinsically disordered region of WRINKLED1 affects its stability and enhances oil accumulation in Arabidopsis. Plant J. 83: 864–874
4. Poliner, E., Panchy, N., Newton, L., Wu, G., Lapinsky, A., Bullard, B., Zienkiewicz, A., Benning, C., Shiu, S.H., Farré, E.M. 2015. Transcriptional coordination of physiological responses in Nannochloropsis oceanica CCMP1779 under light/dark cycles. Plant J. 2015 Jul 27. doi: 10.1111/tpj.12944.
5. Yang Y, Munz J, Cass C, Zienkiewicz A, Kong Q, Ma W, Sanjaya S, Sedbrook JC, Benning C. 2015. Ectopic expression of WRI1 affects fatty acid homeostasis in Brachypodium distachyon vegetative tissues. Plant Phys. pp.01236.2015 (Epub ahead of print).
6. Warakanont J, Tsai CH, Michel EJ, Murphy GR 3rd, Hsueh PY, Roston RL, Sears BB, Benning C. 2015. Chloroplast lipid transfer processes in Chlamydomonas

reinhardtii involving a TRIGALACTOSYLDIACYLGLYCEROL 2 (TGD2) orthologue. Plant J. doi: 10.1111/tpj.13060. (Epub ahead of print).
7. Du Z.-Y., Benning C. 2015. Triacylglycerol accumulation in photosynthetic cells in plants and algae. In: Lipids in Plant and Algae Development. Eds. Nakamura, Y., Li-Beisson, Y. Springer, in press

Rebecca L. Roston, University of Nebraska-Lincoln

Investigations of chloroplast inner membrane and thylakoid connectivity

Objective 1: Understanding architectural dynamics in plant thylakoid membranes

Impact Statement: This year we have successfully established our laboratory and expanded earlier work on developing methods to visualize connectivity events between the thylakoid and inner envelope membrane. We have produced vectors for three visualization strategies and begun to transform them into Arabidopsis. We have demonstrated targeting of control constructs successfully to the inner membrane, as evidenced by fluorescent imaging. We have further developed an isolation procedure for chloroplast vesicles which is promising for isolation of specific stromal vesicles. In collaboration with Dr. Christoph Benning at Michigan State University, we are readying a paper on the sensing of freezing by SFR2, a protein of the chloroplast envelope. More recently, we have begun a collaboration with Dr. Julie Stone at the University of Nebraska-Lincoln to determine the sub-chloroplast location of AtDJ1C.

Accomplishments:

1. Demonstrated targeting of fluorescent proteins to the chloroplast intermembrane space.
2. Production of multiple vectors for fluorescent visualization of chloroplast membrane connectivity.
3. Determination of the freezing sensing mechanism of SFR2, a chloroplast membrane protein.

Plans for Coming Year:

- Determine the sub-chloroplast location of AtDJ1C in collaboration with Dr. Julie Stone's lab.
- Produce Arabidopsis stably expressing the fluorescent visualization constructs in development now.
- Test available approaches to isolate chloroplast vesicles towards the end of isolating vesicles involved in transport between chloroplast membranes.

Publications (2015):

Tsai, C.H., Zienkiewicz, K., Amstutz, C.L., Brink, B.G., Warakanont, J., Roston, R., and Benning, C. (2015). Dynamics of protein and polar lipid recruitment during lipid droplet assembly in Chlamydomonas reinhardtii. Plant J **83,** 650-660.

Vu, H.S., Roston, R., Shiva, S., Hur, M., Wurtele, E.S., Wang, X., Shah, J., and Welti, R. (2015). Modifications of membrane lipids in response to wounding of Arabidopsis thaliana leaves. Plant Signal Behav **10,** e1056422.

Other: Collaborations between Dr. Julie Stone's and Dr. Christoph Benning's lab.

Anastasios Melis, Univ. of California, Dept. of Plant & Microbial Biology, Berkeley, CA

Objective 2: Photosynthetic Capture and Photorespiratory Release of CO_2

Impact Statement:

Bacterial proteins have been heterologously over-expressed in cyanobacteria, up to 15% of total soluble protein, by using the strong *cpc* operon promoter (Kirst et al. 2014; Zhou et al. 2014). However, heterologous expression in cyanobacteria of proteins from higher plants, *e.g.* terpene synthases, does not yield high levels of recombinant protein, even under the control of the same strong *cpc* operon promoter and following the necessary codon-use optimization (Lindberg et al. 2010; Bentley et al. 2013; Formighieri and Melis 2014a; Jindou et al. 2014; Xue et al. 2014; Halfmann et al. 2014). This limitation in the expression of plant genes in cyanobacteria negatively impacts product yield, thus undermining commercial exploitation of these important photosynthetic microorganisms in the generation of plant-based products. This barrier was overcome upon a fusion of transgenic plant proteins to the highly expressed β-subunit of phycocyanin (*cpcB* gene) or to the highly expressed *NPTI* gene in cyanobacteria, which was necessary and sufficient to drive over-accumulation of a recalcitrant plant protein.

Accomplishments:

A fusion protein approach helped to overcome limitation in the expression of recalcitrant plant genes in cyanobacteria, thus improving rates and yield of product generation in these important photosynthetic microorganisms.

Plans for Coming Year:

- We will continue to explore the regulation of plant gene expression and its limitations in cyanobacteria.

Publications (2015):

Formighieri C, Melis A (2015) A phycocyanin•phellandrene synthase fusion enhances recombinant protein expression and β-phellandrene (monoterpene) hydrocarbons production in *Synechocystis* (cyanobacteria). Metab Eng 32:116–124 http://dx.doi.org/10.1016/j.ymben.2015.09.010

Gerald Edwards, WA-AES

Objective 2: Photosynthetic Capture and Photorespiratory Release of CO_2

Impact Statement:
C_4 plants have the most efficient form of photosynthesis on Earth where high losses of CO_2 occur by photorespiration (warm climates, water deficits). With the interest of transferring C_4 traits into crops lacking C_4 photosynthesis, we are studying an unusual form of C_4 which occurs within individual mesophyll cells (unlike the Kranz forms which require cooperative functioning of mesophyll and bundle sheath cells).

Accomplishments:
1. We used a combination of 454 cDNA sequencing and quantitative proteomics in a study of the single-cell C_4 species *Bienertia sinuspersici* to determine the identity and subcellular localization of proteins involved in this unusual mode of carbon fixation. Proteins required to operate a NAD-malic enzyme C_4 cycle between two cytoplasmic domains in mesophyll cells were identified. Analyses of the degree of differentiation of the two chloroplast types for function in C_4 indicate mechanisms exist for selective targeting of nuclear encoded proteins to chloroplasts.

2. During evolution of C_4 photosynthesis, amino acid substitutions in Rubisco and phosphoenolpyruvate carboxylase (PEPC) occurred, with convergence in changes in kinetic properties for optimization in C_4 function. Our analyses of these carboxylases in single-cell C_4 species and sister Kranz type C_4 species in family Chenopodiaceae, show convergence in kinetic properties, while lacking the same mutations found in other C4 systems. Thus, they show during evolution of C_4 there is divergence in amino acid substitutions that alter enzyme kinetics to converge on the same function.

3. Transfer of C_4 traits into C_3 plants requires an understanding of the biochemistry of C_4. Three C_4 cycles occur in C_4 plants which are supported by the decarboxylases, phosphoenolpyruvate carboxykinase (PEPCK), NADP-malic enzyme (NADP-ME), and NAD-malic enzyme (NAD-ME). Recently it has been suggested that mixed decarboxylases systems function within various C_4 species in different families, e.g. PEPCK/NAD-ME or PEPCK/NADP-ME. We evaluated PEPCK by enzyme assay and western blots, along with malic enzymes, in representatives of Poaceae, Aizoaceae, Cleomaceae, and Chenopodiaceae. These results, which support earlier studies, indicated C_4 species can be classified biochemically according to the dominant decarboxylase recruited for C_4 function. Poaceae remains the only family in which PEPCK is known to have a significant role in C_4 photosynthesis.

Plans for Coming Year:
- Characterize the biochemical and structural transitions along a longitudinal leaf gradient in single cell C_4 species which leads to the development of C_4 photosynthesis.

- Complete a study on 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.

Publications:

Offermann, S., Friso, G., Doroshenk, K.A., Sun, Q., Sharpe, R.M., Okita, T.W., Wimmer, D., Edwards, G.E., and van Wijk, K.J. 2015 Developmental and subcellular organization of single-cell C_4 photosynthesis in *Bienertia sinuspersici* determined by large scale proteomics and cDNA assembly from 454 DNA sequencing. J Proteome Research **14**, 2090-2108.

Rosnow, J.J., Evans, M.A., Kapralov, M.V., Cousins, A.B., Edwards, G.E., Roalson, E.H. 2015 Kranz and single-cell forms of C_4 plants in subfamily Suaedoideae show kinetic C4 convergence for PEPC and Rubisco with divergent amino acid substitutions. J Exp Bot doi:10.1093/jxb/erv431

Koteyeva, N.K., Voznesenskaya, E.V., Edwards, G.E. 2015 An assessment of the capacity for phosphoenolpyruvate carboxykinase to contribute to C4 photosynthesis. Plant Science **235,**70-80. doi:10.1016/j.plantsci.2015.03.004

Other:

I had collaborative activities in the NC1200 group this year with Drs. Tom Okita and Asaph Cousins.

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:
Prior to retirement on June 30, 2015, 319 *Chlamydomonas* strains were cataloged and deposited in the *Chlamydomonas* Resource Center (http://chlamycollection.org). Many of the mutants have not been completely characterized, and may be of interest to scientists investigating photosynthesis in general and Rubisco in particular. A detailed list with descriptions is available on request.

(CC-4620 to CC-4640) Rubisco Nuclear-Mutant 76-5EN and Revertants.
(CC-4641 to CC-4650) Rubisco Nuclear Mutants.
(CC-4651 to CC-4665) Phosphoribulokinase (PRK) Mutants and Revertants.
(CC-4666 to CC-4688) CO_2-Concentrating-Mechanism (CCM) Mutants.
(CC-4690 to CC-4701) Hosts for Transformation.
(CC-4702 to CC-4727) Rubisco Activation, Cysteine, and Channel Mutants.
(CC-4728 to CC-4737) Rubisco Small-Subunit A-B-Loop Mutants.
(CC-4739 to CC-4767) Small-Subunit Chimeras, Conserved Residues, and Hybrids.
(CC-4782 to CC-4831) Rubisco Large-Subunit Mutants.
(CC-4832 to CC-4848) Large-Subunit Mutant 68-4PP.
(CC-4849 to CC-4866) Small-Subunit Suppressors of Large-Subunit Mutant 68-4PP.
(CC-4868 to CC-4883) Large-Subunit Mutants (Unfinished).
(CC-4886 to CC-4908) Wild-Type Strains and Chloroplast Photosynthesis Mutants.
(CC-4924 to CC-4935) Foreign Rubisco in Chlamydomonas.
(CC-4936 to CC-4945) Rubisco Posttranslational-Modification Mutants.
(CC-5014 to CC-5040) Rubisco Phylogenetic Engineering.

Plans for Coming Year:
- Prepare several manuscripts for publication.

Publications (2015):
There were no publications this year.

James Moroney, Louisiana State University

Objective 2. Photosynthetic Capture and Photorespiratory Release of CO2

1. Identification of new components of the CCM of Chlamydomonas reinhardtii. In 2011 my laboratory initiated a large scale insertional mutagenesis screen. In this screen we transformed Chlamydomonas reinhardtii with a cassette that confers resistance to paromomycin. We selected for colonies resistant to paromomycin and then screen those colonies for ones that grew well autotrophically on elevated CO2 and grew poorly on low CO2. We then located the genomic site(s) of the insertion using adapter PCR. Some of these results are summarized in the Jungnick et al. (2014) publication listed below. This past year we have looked at the pyrenoids of some of them using visible microscopy as well as electron microscopy. We have found a few of the mutants that do not grow well on low CO2 also show either abnormal pyrenoids or abnormal starch deposition. Normally, in wild-type cells, a starch sheath forms around the pyrenoid when cells are grown on low CO2. We are also investigating a mutant where the paromomycin insert has inserted into a transporter of the solute carrier class. This mutant grows poorly on low CO2 and shows reduced uptake of inorganic carbon at high pH where bicarbonate is the predominate species of inorganic carbon. Our current hypothesis is that this transporter either directly participates in inorganic carbon uptake or is indirectly required because it transports some other ion required for cotransport or counter transport at high external pH.

2. Which Arabidopsis thaliana carbonic anhydrase isoforms assist in the delivery of CO2 to Rubisco? Arabidopsis has 14 carbonic anhydrase genes and we have been systematically obtaining lines missing one or more of the genes to determine which, if any, are involved in delivery of CO2 to Rubisco. We focused first on the carbonic anhydrases found in the cytoplasm. We have obtained knockout lines for .CA2 and .CA4. .CA4 has two transcriptional start sites, one produces a protein that localizes to the plasma membrane and the other produces a protein that is cytoplasmic. .CA2 is cytoplasmic. Plants missing .CA2 or .CA2 individually grow normally, but plants missing both .CA2 and .CA4 grow slowly under low CO2 conditions. The double knock out plants grow normally on elevated CO2 (1200 ppm CO2). Right now we are finishing up characterizing this double mutant by looking at key metabolites as well as measuring its photosynthetic parameters.

We have also begun work on the carbonic anhydrases found in the chloroplast. Two carbonic anhydrase isoforms are clearly in the chloroplast. .CA1 is a very abundant carbonic anhydrase comprising almost 1% of the soluble protein in leaves. .CA5 is also found in the chloroplast and its expression appears to be limited to only a few tissues. When we investigated the growth phenotypes of plants missing either .CA1 or .CA5 we were quite surprised to see that plants missing .CA1 showed no growth reduction even under low CO2 conditions while .CA5 knockout plants were barely viable at ambient CO2 levels. .CA5 knockout plants can grow under elevated CO2 conditions but are still not normal. We are currently investigating the expression of each of these genes in the plant. In addition, we suspect that one or more of the other carbonic anhydrase genes

also encode chloroplastic carbonic anhydrase isoforms and we are using GFP chimeras to determine which of the other genes encodes an isoform that is targeted to the chloroplast. We suspect that one of these other genes might encode a carbonic anhydrase that is redundant in function with .CA1 and this might explain why the .CA1 knockout plant grows normally at low CO2 concentrations.

Usefulness of the Results

The carbon dioxide concentrating mechanism of algae is an adaptation to limiting CO2 conditions. Aquatic photosynthetic organisms must deal with low and variable CO2 levels in the water. In addition, the diffusion of CO2 in water is thousands of times slower than the diffusion of CO2 in air. As a result aquatic organisms often deplete the CO2 around them and this CO2 pool must be replenished from other forms of inorganic carbon, notably bicarbonate. The first aim of this research project was to discover how algae accomplish this uptake of CO2 and how they enhance photosynthesis when the CO2 concentration is low. The long-term goal of this work is to identify components of the CCM that might potentially be transferred from algae to crop plants to improve photosynthesis and crop yield. The second aim of this project is to determine which carbonic anhydrases are facilitating the delivery of CO2 to Rubisco in C3 plants. At this point we know that eliminating the cytoplasmic carbonic anhydrases, .CA2 and .CA4, reduces growth on limiting CO2. In addition loss of .CA5 is highly detrimental to the plant. We are now working to determine whether the loss of these carbonic anhydrases affects photosynthesis or some other metabolic pathway. The findings of this project not only increase our knowledge of photosynthesis, but also identify algal components of the CCM that might aid in improving photosynthesis in crop plants in the future.

Plans for the Upcoming Year

1. We are characterizing the new high CO2-requiring C. reinhardtii strains that have abnormal pyrenoid morphologies.
2. We are characterizing the new high CO2-requiring C. reinhardtii strain that has a disrupted and uncharacterized transport protein.
3. We will finish our characterization of the .CA2 .CA4 Arabidopsis double mutant which we have shown is missing all of the cytoplasmic carbonic anhydrases.
4. We will continue our work on the chloroplast carbonic anhydrases of Arabidopsis.

Publications in 2014

Moroney, J.V. Wee J.L. 2014. CCM8: The Eighth International Symposium on Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. Photosynth. Res. 121:107-110. http://link.springer.com/article/10.1007/s11120-013-9965-4/fulltext.html

Jungnick, N., Ma, Y., Mukherjee, B., Cronan, J.C., Speed, D.J., Laborde, S.M., Longstreth, D.J., Moroney J.V. 2014. The Carbon Concentrating Mechanism in Chlamydomonas reinhardtii - Finding the Missing pieces. Photosynth. Res. 121:159-173 http://link.springer.com/article/10.1007/s11120-014-0004-x/fulltext.html

Memmola, M., Mukherjee, B., Moroney, J.V., Giordano M. 2014. Carbon allocation and element composition in four Chlamydomonas mutants defective in genes related to the CO2 Concentrating Mechanism. Photosynth. Res. 121:201-211 http://link.springer.com/article/10.1007/s11120-014-0005-9/fulltext.html

Steven C. Huber, Illinois AES/ARS

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Impact Statement: Insights about the regulatory mechanisms controlling signaling pathways and metabolic enzymes 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. Rubisco is the CO2-fixing enzyme of the Calvin-Benson Cycle upon which all plant and crop productivity is dependent and Rubisco activity requires the continual 'molecular chiropractic activity' of its helper protein, activase. Rubisco activase is known to be controlled by redox regulation (reversible oxidation/reduction of two cysteine residues) such that its activity fluctuates with changes in light and as a result Rubisco activity changes accordingly. Recent results from our laboratory indicate that activase is phosphorylated on a specific residue (threonine-78) when leaves are darkened and thus phosphorylation emerges as a new biochemical mechanism that may contribute to control of activase activity. The trigger for dark-induced phosphorylation of activase appears to be oxidation of stromal thioredoxin(s) that activate the protein kinase, cpCK2α, and/or inhibit the (presently unidentified) protein phosphatase(s). Experiments with the cpck2 knockout suggest that phosphorylation of Thr78 is not required for control of activase when redox regulation is present, but further studies are required to determine whether it plays a redundant role in regulation. Identifying post-translational modifications provides an opportunity to manipulate pathways using genome editing technologies and thus have impact on both science and technology. (Collaboration with M. Spalding).

2. An autophosphorylation database was developed for the Arabidopsis LRR RLK family that will serve as a useful entry point for family-wide functional analysis of receptor kinase phosphorylation. Many of the LRR RLKs in this database have known functions and are actively being investigated by numerous laboratories. The data generated here may guide site-directed mutagenesis of specific phosphorylation sites followed by expression in a LRR RLK mutant with a visible phenotype to determine the extent of rescue to wild type, which is a standard approach for analyses of LRR RLK functional phosphorylation sites *in planta.* Furthermore, knowledge of specific phosphorylated tryptic peptides provides a resource for planning highly sensitive selected reaction monitoring LC-MS/MS experiments, particularly with a triple quadrupole mass spectrometer, which greatly increases ability to detect *in vivo* phosphorylation sites in targeted LRR RLK studies. Finally, synthetic peptides derived from our dataset could be used to generate phosphorylation-specific antibodies to monitor potential *in vivo* phosphorylation sites by immunoblot analysis.

3. Unexpected binding of calmodulin to CDPKs. Interaction of calcium/calmodulin has been confirmed with several plant protein kinases using calmodulin overlay assays and real-time protein:protein interactions with several receptor kinases and calcium-

dependent protein kinases (CDPKs). Differences in binding affinity among protein kinases was established confirming the specificity of the assays. In more detailed studies with CPK28, autophosphorylation of the kinase did not affect binding to calmodulin. Preliminary results suggest that the calmodulin binding resides in the carboxy-terminal portion of the protein beyond the kinase domain. These results provide the foundation for future studies to determine the functional role of calcium/calmodulin binding to protein kinases.

Plans for Coming Year:

- In collaborative studies with M. Spalding (ISU) will analyze transgenic rice with targeted editing of the Rubisco activase gene to further test the hypothesis that maintaining Rubisco activation state at low light enhances photosynthesis and plant growth in a rapidly changing low light / high light environment.
- Use RNAi to down regulate expression of soybean *RCA* alpha-subunit genes to translate results from Arabidopsis to soybeans growing in the field.
- Identify interacting proteins with the receptor kinase, BRI1 in Arabidopsis.
- Conduct an expanded screen for tyrosine phosphorylation activity across the plant receptor kinase family in conjunction with functional studies of tyrosine phosphosites with specific receptor kinases that have known function.

Publications (2015):

Bender, K. W., Wang, X., Cheng, G.B., Kim, H.S., Zielinski, R.E., and Huber, S.C. (2015). Glutaredoxin AtGRXC2 catalyzes inhibitory glutathionylation of Arabidopsis BRI1-ASSOCIATED RECEPTOR-LIKE KINASE 1 (BAK1) in vitro. Biochem. J. 467: 399-413**.**

Mitra, S.K., Chen, R., Dhandaydham, M., Wang, X., Blackburn, R.K., Kota, U., Schwartz, D., Huber, S.C. and Clouse, S.D. (2015). An autophosphorylation database for leucine-rich repeat receptor-like kinases in Arabidopsis thaliana. Plant J. 82: 1042-1060 DOI: 10.1111/tpj.12863

Oh, M.-H., Bender, K.W., Kim, S.Y., Wu, X., Lee, S., Nou, I.-S., Zielinski, R.E., Clouse, S.D. and Huber, S.C. (2015). Functional analysis of the BRI1 receptor kinase by Thr-for-Ser substitution in a regulatory autophosphorylation site. Front. Plant Sci., 10.3389/fpls.2015.00562

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. Our research will help understand sources and sinks for glucose 6-phosphate, which may determines the rate of the glucose 6-phosphate shunt that bypasses much of the Calvin-Benson cycle.

Accomplishments:

1. Arabidopsis plants expressing the gene for a glucose-6-phosphate transporter (GPT2) were grown. This transporter is normally only expressed in leaves with excess sugar, for example leaves fed sucrose. Unfortunately, this gene is getting silenced effectively slowing our progress.
2. A hypothesis was developed that G6P used for starch synthesis can also be used in the reductive pentose phosphate pathway making a futile cycle around the Calvin-Benson cycle. This increases the required ratio of ATP to NADPH, increasing cyclic electron flow. Increased cyclic electron flow has been demonstrated.

Plans for Coming Year:

- Metabolomics – Measure G6P levels in the stroma by non-aqueous fractionation
- Determine the properties of PGI and glucose-6-phosphate dehydrogenase (G6PDH) – There are preliminary indications that these enzymes have unusual properties that regulate the G6P shunt. Activities will be determined in proteins made from plant genes expressed in *E. coli*.
- Engineer plants with unregulated PGI – This will test whether PGI is kinetically limiting during photosynthesis and what are the consequences when too much F6P is converted to G6P.
- Finding the slow labeling pool of carbon – We will test the hypothesis that maltodextrin made during starch synthesis is the slow-to-label carbon pool.

Publications (2015):

Sharkey, T.D. (2015a). What gas exchange data can tell us about photosynthesis. Plant Cell Environ. **in press**.

Sharkey, T.D. (2015b). Understanding carbon partitioning and its role in determining plant growth Plant Cell Environ. **in press**.

Weise, S.E., Carr, D.J., Bourke, A.M., Hanson, D.T., and Sharkey, T.D. (2015). The *arc* mutants of *Arabidopsis* with fewer large chloroplasts have a lower mesophyll conductance. Photosynth. Res. **124,** 117-126.

Weraduwage, S.M., Chen, J., Anozie, F.C., Morales, A., Weise, S.E., and Sharkey, T.D. (2015). The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Frontiers in Plant Science **6,** 167.

Karen E. Koch, University of Florida

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Impact Statement:

New avenues for increasing grain yield have been identified that enhance photosynthate movement into these harvested structures by either 1) enhanced expression of SWEET-type hexose transporters at the base of the grain (increased seed size during domestication of rice and maize), 2) targeted alteration of sugar-signals at pollination using site-specific alterations in trehalose metabolism (increased kernel number), and 3) changes in strigolactone biosynthesis and/or signal transduction (domestication-based increases in seed size).

Accomplishments:

1. We demonstrated that a dramatic, defective-kernel phenotype arose from a mutation in the *SWEET4* hexose transporter of maize, and determined that its primary site of action was in the basal endosperm transfer layer (BETL) of kernels. Together with collaborators (Sosso et al., 2015), the roles of this gene and its counterpart in rice were found to be associated with both seed size and domestication. Two mutant SWEET4 alleles with the same kernel phenotype in maize confirmed that this gene was essential for delivery of the photosynthates required for growth of a kernel (Sosso et al., Nature Genetics, 2015).

2. In the process of appraising results from targeted alterations in trehalose metabolism employed by Nuccio et al., (2015) to enhance yield, we expanded proposed mechanisms for effects on sugar signals and photosynthate delivery (Guan and Koch, Nature Biotech, 2015).

3. We found that kernel size is constrained in a strigolactone-deficient maize mutant (*zmccd8*) by domestication-related physical features of the female floral structures (Guan and Koch, new data).

4. We generated, mapped, and sequenced insertion sites for 7,822 new maize mutants for public use. Many photosynthetic and partitioning mutants are among these. They can be searched by sequence and requested at MaizeGDB.org.

Plans for Coming Year:

- We will test roles of other maize genes that affect photosynthate import into maize kernels. Focus will be directed toward phloem-unloading zones, post-phloem transport, and mechanisms of deposition in endosperm.

- We will further explore possible mechanisms underlying the role of strigolactone in kernel size and maize domestication.

- We will continue to generate, identify, and characterize maize mutants with potential to alter photosynthate partitioning and sugar sensing.

Publications (2015):

Guan J.C., and Koch, K.E. (2015) A time and a place for sugar in your ears: Targeting trehalose metabolism improves maize yield under a range of water-deficit conditions. Nature Biotechnology **33,** 827-828.

Sosso, D. Luo, D., Li, Q-B., Sasse, J., Gendrot, G., Suzuki, M., Koch, K.E., McCarty, D.M. Chourey, P.S., Rogowsky, P.M., Ross-Ibarra, J., Yang, B., and Frommer, W.B. (2015) Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. Nature-Genetics. On-line Advance Pub, Nov 2, 2015 **DOI: 10.1038/ng.3422.**

Djidonou, D., Lopiano, K., Zhao, X., Simonne, E.H., Erickson, J.E., and Koch, K.E. (2015) Estimating nitrogen nutritional crop requirements of grafted tomatoes under field conditions. Scientia Horticulturae. **182,** 18-26.

Andorf, C.M., Kopylov, M., Dobbs, D., Koch, K.E., Stroupe, M.E., Lawrence, C.J., and Bass, H. (2014) G-quadruplex (G4) motifs in the maize (*Zea mays* L.) genome are enriched at specific locations in thousands of genes coupled to energy status, hypoxia, low sugar, and nutrient deprivation. JGG (J. Genetics and Genomics). **41,** 627-647.

Hunter C.T., Suzuki M., Saunders J.W., Wu S., Tasi A., Avigne W.T., McCarty D.R., and Koch K.E. (2014) Phenotype to genotype using forward-genetic Mu-seq for identification and functional classification of maize mutants. Frontiers in Plant Science. **4,** 545. DOI: 10.3389/fpls.2013.00545.

Other:

- National Secretary and Program Chair for the American Society for Plant Biology (Elected).
- HHMI Finalist for national award: Outstanding International Graduate Student (Peng Liu, Koch lab, UF)

Mike Giroux, Department of Plant Sciences, Montana State University

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.

2015 Accomplishments:

1. Determined that an increase in both seed starch and leaf starch together is associated with greater yield advantages over either increased leaf, or seed starch alone.
2. Completed photosynthetic rate studies comparing the impact of increased leaf and/or seed starch upon whole plant growth. Interestingly, there was no change in the rate of photosynthesis in any genotype. This is interesting as it implies that the yield advances observed in the yield trial were achieved via a mechanism not involving photosynthesis on a per unit basis.
3. The leaf and seed starch biosynthetic transgenes resulted in significantly higher leaf starch by the end of the photoperiod and alter leaf carbon metabolism and gene expression.

Plans for Coming Year:

- Complete gene expression experiments by examining expression of genes known to be important to carbon metabolism.
- Examine global changes in gene expression with annotation clustering of genes significantly up and downregulated in each genotype.
- Summarize metabolomic study results.
- Complete and submit a paper for publication.
-

Publications (2014):

Schlosser A.J., Martin J.M., Beecher B.S., Giroux M.J. (2014) Enhanced rice growth is conferred by increased leaf ADP-glucose pyrophosphorylase activity. Journal of Plant Physiology & Pathology 2:4.

Thomas W. Okita, WA-AES

Objective 3: ***Mechanisms Regulating Photosynthate Partitioning.***

Impact Statement:
The sink strength of developing cereal grains is dictated by the conversion of transported photoassimilates into starch and protein, the major storage reserves. Current efforts have been directed at understanding how starch and proteins are synthesized and stored in developing rice seeds. Specifically, we are interested in elucidating the role of the regulatory enzymes, ADPglucose pyrophosphorylase (AGPase) and starch phosphorylase 1 (Pho1) in starch biosynthesis and how storage protein synthesis is controlled by the localization of RNAs on distinct subdomains of the cortical endoplasmic reticulum.

Accomplishments:
Using isothermal titration calorimetry, the wildtype heterotetrameric ($S^{WT}L^{WT}$) AGPase was shown to possess two distinct ATP binding sites, whereas the homotetrameric LS and SS variant forms only exhibited properties of one of the two binding sites.
EM1093 rice line which produced shrunken seeds was found to contain a mutation in the Brittle1 gene, which codes for the ADPglucose transporter. Analysis of wildtype and ADPglucose-overproducing rice lines expressing OsBt1 indicate that BT1 transport activity does not limit starch synthesis.
RNAseq analysis of transgenic rice lines over-producing ADPglucose and, in turn, increase starch levels, show significant re-programming of gene expression with a significant increase in RNA levels of an uncharacterized starch binding domain protein (SBDP) and reduction in RNA levels for starch synthase III. SBDP was demonstrated to interact with SSIII.
The RNA binding protein, RBP-P, which interacts with the glutelin zipcode sequence was found to bind directly to NSF and three other RNA binding proteins and indirectly to Rab5. This evidence provides direct evidence for the involvement of membrane trafficking events in RNA localization.

Plans for Coming Year:
Continue studies to identify the constraints that limit maximum carbon flow into starch in the rice lines over-producing ADPglucose
Continue studies to identify proteins involved in RNA transport and localization and the sets of RNAs that are targeted by these proteins.

Publications (2015):
Cakir, B., Tuncel, A., Hwang, S.-K., and Okita, T.W. (2015a). Increase of grain yields by manipulating starch synthesis. In Starch: Metabolism and Structure, Y. Nakamura, ed (Japan: Springer), pp. 371-395.
Cakir, B., Tuncel, A., Green, A.R., Koper, K., Hwang, S.-K., Okita, T.W., and Kang, C.-H. (2015b). Substrate binding properties of ADP-glucose pyrophosphorylase determined by isothermal titration calorimetry. FEBS Lett. **589,** 1444-1449.

Cakir, B., Shiraishi, S., Tuncel, A., Matsusaka, H., Satoh, R., Singh, S., Hwang, S.-K., Satoh, H., and Okita, T.W. (2015c). Analysis of the rice ADPglucose transporter (OsBT1) indicates the presence of regulatory processes in the amyloplast stroma that control ADPglucose flux into starch. submitted.

Hwang, S.-K., Singh, S., Satoh, H., and Okita, T.W. (2015). Plastidial starch phosphorylase from the starch storage tissue of rice is highly active at low temperature. . Submitted.

Offermann, S., Frisco, G., Doroshenk, K.A., Sun, Q., Sharpe, R.M., Okita, T.W., Edwards, G.E., and van Wijk, K.J. (2015). Development and subcellular organization of single-cell C4 photosynthesis in Bienertia sinuspersici determined by large scale proteomics and cDNA assembly from 454 DNA sequencing. J. Proteome Res. **14,** 2090-2108.

John C. Cushman, Nevada AES

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Impact statement:

- Microalgae offer great potential as a third-generation biofuel feedstock, especially when grown on wastewater, as they have the dual application for wastewater treatment and as a biomass feedstock for biofuel production. Highly concentrated wastewater can be used to grow microalgae, which limits the need to dilute wastewater prior to algal production.

- Reiterative, transgressive selection strategies using buoyant density gradient centrifugation and fluorescence-activated cell sorting and flow cytometry represent potential alternatives to genetic engineering strategies for the alteration of microbial biofuel feedstock traits.

Accomplishments:

1. The potential for growth on wastewater centrate was evaluated for forty microalgae strains from fresh (11), brackish (11), or saltwater (18) genera. Freshwater strains were able to grow at high concentrations of centrate, with two strains, *Neochloris pseudostigmata* and *N. conjuncta*, demonstrating growth at up to 40% v/v centrate (Hiibel et al., 2015).
2. Buoyant density gradient centrifugation and fluorescence-activated cell sorting and flow cytometry have been used successfully to select and isolate algal strains with greater starch or lipid contents (Hathwaik et al., 2015).

Plans for the Coming Year:

- Complete studies on transcriptome profiling of saltwater algae undergoing nutrient deprivation.

Objective 4: Developmental and Environmental Limitations to Photosynthesis.

Impact statement(s):

- The introduction of bioengineered Crassulacean acid metabolism (CAM) into short-rotation forestry bioenergy trees is a potentially viable path to sustaining agroforestry production systems in the face of a globally changing climate.
- *Agave* and *Opuntia* represent highly water-use efficient bioenergy crops that are less recalcitrant to deconstruction than are traditional lignocellulosic biomass feedstocks and possess high water contents, which might prove advantageous for biomass deconstruction processes as less input water would be needed.
- *Agave* and *Opuntia* represent highly water-use efficient bioenergy crops that are useful for diversifying the bioenergy feedstock portfolio because they are suitable for expanding feedstock production into semi-arid, abandoned, or degraded agricultural lands and potentially reclaim drylands.
- CAM is a specialized mode of photosynthesis that features nocturnal CO_2 uptake, facilitates increased water-use efficiency (WUE), and enables CAM plants to inhabit

water-limited environments. Current research is focused on characterizing the genome-scale requirement of CAM using comparative genomics approaches with an evolutionary framework of taxonomically diverse CAM species.

Accomplishments:

1. One approach to sustaining plant productivity is to improve water-use efficiency by engineering CAM into C_3 photosynthesis crops such as fast-growing, short-rotation forestry bioenergy crops such as poplar (*Populus* spp.) and willow (*Salix* spp.), which are particularly susceptible to hydraulic failure following drought stress (Borland et al., 2015).
2. The biomass composition of *Agave tequilana* and *Opuntia ficus-indica* was analyzed and had lower lignin contents and crystalline cellulose contents, indicating that these biomass feedstocks would be far less recalcitrant to deconstruction than are traditional woody, lignocellulosic biomass feedstocks (Yang et al., 2015).
3. Although *Agave* and *Opuntia* represent highly water-use efficient bioenergy crops, detailed ground truth studies about the productivity of these alternative bioenergy crops are needed (Cushman et al., 2015). The Cushman lab has initiated such a field study in Logandale, NV examining the biomass production of three *Opuntia* species under three different irrigation regimes.
4. Genome-scale information from transcriptome and genome sequencing is being collected from a variety of key CAM species (Yang et al., 2015) such as pineapple, an agronomically important CAM species (Ming et al., 2015). Such information is being used to understand CAM evolution and to move CAM into C_3 photosynthesis food and bioenergy crops thereby improving their water-use efficiency.

Plans for the Coming Year:

- Complete the genome sequencing of the common ice plant *(Mesembryanthemum crystallinum* L.), a facultative CAM species. Detailed molecular analysis of a thick-leaf mutant and an epidermal bladder cell-less mutant of ice plant will also be completed.
- Complete transcriptome sequencing of the prickly pear cactus *(Opuntia ficus-indica*), an octoploid, obligate CAM species.
- Complete the transcriptome and genome sequencing of the prickly pear cactus *(Opuntia cochenillifera*), a diploid, obligate CAM reference species.
- Complete the transfer of the carboxylation, decarboxylation, and stomatal control modules of CAM into C_3 model (*Arabidopsis*). Work towards moving these same CAM modules into *Populus*, a SRF bioenergy crop.
- Continue the analysis of large CAM species, such as *Opuntia* and *Agave*, to investigate their potential use as feedstocks for food and fodder, and technologies for their efficient conversion to biofuel precursors.

Publications (2015):

Borland, A.M., Wullschleger, S.D., Weston, D.J., Tuskan, G.A., Hartwell, J., Yang, X., Cushman, J.C. (2015) Climate-resilient agroforestry: Physiological responses to climate change and engineering of crassulacean acid metabolism (CAM) as a mitigation strategy. Plant Cell & Environ. **38**,1833-1849. DOI:10.1111/pce.12479.

Cushman, J.C., Davis, S.C., Yang, X., Borland, A.M. (2015) Development and use of bioenergy feedstocks for semi-arid and arid lands. Journal of Experimental Botany. **66**,4177-4193. DOI: *10.1093/jxb/erv087.*

Hathwaik, L.T., Redelman, D., Samburova, V., Zielinska, B., Shintani, D.K., Harper, J.F., Cushman, J.C. (2015) Transgressive, reiterative selection of *Dunaliella salina* with enhanced lipid and starch production using continuous buoyant density gradient centrifugation. Algal Research. **9**, 194-203.

Hiibel, S.R., Lemos, M.S., Kelly, B.P., Cushman, J.C. (2015) Microalgae biofuel feedstock production using municipal wastewater. Frontiers in Energy Research. **3**, 1-8. doi: 10.3389/fenrg.2015.00020

Yang, L., Carl, S., Lu, M., Mayer, J.A., Cushman, J.C., Tian, E., Lin, H. (2015) Biomass characterization of *Agave* and *Opuntia* as potential biofuel feedstocks. Biomass and Bioenergy. **76**, 43-53. DOI: 10.1016/j.biombioe.2015.03.004

Yang, X., Cushman, J.C., Borland, A.M., Edwards, E.J., Wullschleger, S.D., Tuskan, G.A., Owen, N.A., Griffiths, H., Smith, J.A.C., De Paoli, H.C., Weston, D.J., Cottingham, R., Hartwell, J., Davis, S.C., Silvera, K., Ming, R., Schlauch, K.A., Abraham, P., Stewart, J.R., Guo, H-B., Albion, R.A., Ha, J., Lim, S.D., Wone, B.W.M., Yim, W.C., Garcia, T., Mayer, J.A., Petereit, J., Nair, S.S., Casey, E., Hettich, R.L., Ceusters, J., Ranjan, P., Palla, K.J., Yin, H., Reyes-García, C., Andrade, J.L., Freschi, L., Beltran, J.D., Dever, L.V., Boxall, S.F., Waller, J., Davies, J., Bupphada, P., Kadu, N., Winter, K., Sage, R.F., Aguilar, C.N., Schmutz, J., Jenkins, J., Holtum, J.A.C. (2015) A roadmap for research on crassulacean acid metabolism to enhance sustainable food and bioenergy production in a hotter, drier world. New Phytologist. **207***,* 491–504.

Ming, R., Van Buren, R., Wai, C.M., Tang, H., Schatz, M.C., Bowers, J.E., Lyons, E., Wang, M-L., Chen, J., Biggers, E., Zhang, J., Huang, L., Zhang, L., Miao, W., Zhang, J., Ye, Z., Miao, C., Lin, Z., Wang, H., Zhou, H., Yim, WC., Priest, HD., Zheng, C., Woodhouse, M., Edger, P.P., Guyot, R., Guo, H-B., Guo, H., Zheng, G., Singh, R., Sharma, A., Min, X., Zheng, Y., Lee, H., Gurtowski, J., Sedlazeck, F., Harkess, A., McKain, MR., Liao, Z., Fang, J., Liu, J., Zhang, X., Zhang, Q., Hu, W., Yuan, Q, Wang, K., Chen, L-Y., Shirley, N., Lin, Y-R., Liu, L-Y., Hernandez, AG., Wright, C.L., Bulone, V., Tuskan, G.A., Heath, K., Zee, F., Moore, P.H., Sunkar, R., Leebens-Mack, J.H., Mockler, T., Bennetzen, J.L., Freeling, M., Sankoff, D., Paterson, A.H., Zhu, X., Yang, X., Smith, J.A.C., Cushman, J.C., Paull, R.E., Yu, Q. (2015) The pineapple genome and the evolution of CAM photosynthesis. Nature Genetics. *In press.*

Other: *Optional (e.g.*, patents, awards, etc.)
Collaborations: The Cushman lab is collaborating with the Harper lab in improving the agronomic traits of *Camelina sativa* by manipulating photosynthate partitioning resulting in increased seed production and oil content using various approaches.

Jeffrey F. Harper, Department of Biochemistry, University of Nevada, Reno NV

Objective 3: Mechanisms Regulating Photosynthate Partitioning

Objective 4: Developmental and environmental limitations to photosynthesis.

Impact Statement: ***The long-term goal is*** to understand a fundamental aspect of sexual reproduction in flowering plants – how pollen tubes grow, locate ovules, and discharge sperm to fertilize egg cells. This knowledge is expected to guide precision breeding strategies to make pollen more tolerant to hot and cold temperature stresses. One of the major threats of climate change is the potential that extreme weather events will limit or reduce yields from important seed crops, such as corn. Because pollination is vulnerable to temperature stresses, developing strategies to improve pollen fertility under stress conditions is critical to our ability to sustain or increase agricultural productivity.

Research in the Harper lab uses Arabidopsis as a model system, and is focused on the structure and biological functions of genes such as calcium pumps (ACAs), calcium-dependent protein kinases (CPKs), cyclic nucleotide gated channels (CNGCs), lipid flippases (ALAs), 14-3-3s, Myb and CAMTA transcription factors, and micro-RNAs. A key hypothesis guiding our research is that cell growth programs and responses to the environment trigger specific information-rich Ca^{2+} signals, some of which are decoded by CPKs to coordinate multiple cellular processes, such as transcription, turgor pressure, the secretion of new cell wall materials, and feedback between cell-wall structures and the growth machinery within the cytoplast.

Accomplishments:

1. A pollen transcriptome (RNA-seq) comparison has been done for wild type and a heat-sensitive knockout mutant, *cngc16* (cyclic nucleotide gated channel 16). The purpose was to identify genes of potential importance to a pollen heat-stress response. Of the 2613 heat-stress dependent changes in wild type pollen, 627 did not occur in the heat-sensitive mutant. This supports an hypothesis that the temperature sensitivity of *cngc16* pollen is at least partly due to a disruption of the pollen's ability to reprogram the transcriptome for a normal heat-stress response.
2. A suppressor mutation has been identified that reverses a near-sterile pollen phenotype for a *cpk17/34* knockout (Ca-dependent protein kinase 17 and 34). The *cpk17/34* knockout results in pollen tubes that are short, slow and unable to locate ovules. The suppressor restores near normal pollen tube growth and fertility, and corresponds to a loss-of-function mutation in a gene encoding an enzyme predicted to function in acetylating cell wall polysaccharides. This supports a working model in which one of the primary functions of CPK17/34 is to regulate a cell wall biogenesis pathway. When this pathway is not properly regulated, the cell wall rigidity is compromised, leading to an attenuation of pollen tube growth.

Plans for Coming Year:

- Test candidate stress-tolerance genes for their ability to be used to improve tolerance of pollen to hot days and cold nights.
- Develop and test new traits to improve the yield, oil quality, and stress tolerance in *Camelina*, an oil seed crop plant of potential economic importance for dry land agriculture.

Publications (2015):

McDowell, SC, RL Lòpez-Marquès, T Cohen, E Brown, A Rosenberg, MG. Palmgren, JF. Harper (2015). Loss of the *Arabidopsis thaliana* Lipid Flipases ALA6 and 7 alter the lipid composition of pollen and impair pollen tube tip growth. Front Plant Sci. 21:197

Poulsen, RL, López-Marqués, P Pedas, S McDowell, E Brown, R Kunze, JF Harper, TG Pomorski and MG Palmgren (2015). A phospholipid uptake system in the plant Arabidopsis thaliana. Nature Communications 6:7649

Loraine AE, Blakley IC, Jagadeesan S, Harper J, Miller G, Firon N. (2015). Analysis and visualization of RNA-Seq expression data using RStudio, Bioconductor, and Integrated Genome Browser. Methods Mol Biol. 1284:481-501

Glenda E. Gillaspy, Virginia Agricultural Experiment Station (VAES)

Objective 3. Mechanisms regulating photosynthate partitioning

Impact Statement: Glenda Gillaspy (VT) has investigated the mechanisms that regulate sensing of energy status in plants. The response to low energy impacts photosynthate partitioning into biosynthetic pathways, related to Objective 3 of the project. There are two targets for controlling energy responses in plants that we are pursuing. The first is a group of potential low energy signaling molecules called inositol pyrophosphates. We previously reported the characterization of two plant kinase genes (VIP1 and VIP2) involved in inositol pyrophosphate synthesis, and showed that plants accumulate about 1-2 % of their inositol phosphate pool as pyrophosphates. During this past year we have characterized *vip* double mutants and their response to low energy conditions. In addition, we have measured changes in inositol phosphates and pyrophosphates that occur in different energy conditions. Our second target for controlling energy status in plants is the sucrose non-fermenting related kinases (SnRKs), which are major energy sensors in eukaryotes that impact global transcription programs, stress signaling and lifespan. Work on the functional consequences of expression of different SnRK1 genes and protein isoforms was reported last year and shows that specific SnRK1 isoforms increase biomass production later in development.

Accomplishments:

1. Characterized *vip* double mutant responses to different energy conditions.
2. Measured inositol phosphate and pyrophosphate accumulation in plants grown in different energy conditions.
3. Localized VIP and other inositol phosphate pathway enzymes to organellar sites.
4. Measured inositol phosphates in cotton and delineated an unusual accumulation of $InsP_5$.

Plans for Coming Year:

- Refine observed changes in inositol phosphates and energy status.
- Investigate whether inositol phosphates accumulate in organelles.
- Examine metabolic changes in *vip* and SnRK1.1 plants.

Publications (2015):

Phillippy BQ, Perera IY, Donahue JL, and Gillaspy GE (2015) Certain Malvaceae Plants Have a Unique Accumulation of *myo*-Inositol 1,2,4,5,6 Pentakisphosphate. Plants 4: 267-283; doi:10.3390/plants4020267

Williams, S, Gillaspy, G and Perera, I (2015) Biosynthesis and possible functions of inositol pyrophosphates in plants. Front. Plant Sci., doi: 10.3389/fpls.2015.00067

Yen J, Tanniche, I, Fisher, A, Gillaspy, G, Bevan, D, and Senger, R (2015) Designing metabolic engineering strategies with genome-scale metabolic flux modeling. Advances in Genomics and Genetics, 5:93-105.

Nourbakhsh, A, Collakova, E, and Gillaspy, G (2015) Characterization of the Inositol Monophosphatase Gene Family in Arabidopsis. Front Plant Sci. doi: 10.3389/fpls.2014.00725

JC Jang, Ohio State University

Objective 3: Mechanisms Regulating Photosynthate Partitioning
Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:
Plant Arginine-Rich (RR)-Tandem CCCH Zinc Finger proteins (RR-TZFs) are potent regulators of hormone- and environmental cue-mediated plant growth and stress responses. Genetic studies have indicated that RR-TZF proteins can control plant size, flowering time, and enhance biotic and abiotic stress responses via regulation of gene expression. Despite growing evidences, the underlying molecular mechanisms are obscured. We have demonstrated: 1) RR-TZF proteins are conserved across higher plant species; 2) RR-TZFs can target and degrade specific mRNAs; 3) RR-TZFs preferentially interact with stress responsive regulators.

Accomplishments:
1. Demonstrate that Arabidopsis TZF1 binds AU-rich RNA elements via a unique RNA binding domain.
2. Arabidopsis TZF1 can target specific mRNAs for degradation.
3. Identification of Arabidopsis TZF proteins interacting partners.
4. Structure-function analysis of plant RR-TZF proteins.

Plans for Coming Year:
- Genome-wide identification of Arabidopsis TZF1 mRNA targets
- Molecular characterization of Arabidopsis TZF1-RNA interaction
- P-body and stress granule dynamics under biotic and abiotic stresses
- Characterization of components in TZF-dependent signaling pathways

Publications (2014-2015):
1. Bogamuwa, S. and Jang, JC (2014). Tandem CCCH zinc finger proteins in plant growth, development, and stress response. ***Plant and Cell Physiology*** 55: 1367–1375. (Invited Review).
2. Qu, J., Kang, S. G., Musier-Forsyth, K., and Jang J. C. (2014). *Arabidopsis thaliana* tandem zinc finger 1 (AtTZF1) in RNA binding and decay. ***Plant Journal*** 78: 452-467.
3. Qu. J., Kang, S. G., Hah, C., and Jang, J.-C. (2015). Molecular and Cellular Characterization of GA-Stimulated Transcripts GASA4 and GASA6 in *Arabidopsis thaliana.* ***Submitted.***
4. Bogamuwa, S. and Jang, JC (2015). Plant tandem CCCH zinc finger proteins interact with ABA, drought, and stress response regulators in P-bodies and stress granules. ***Submitted****.*
5. Lin, P. C., Qu, J., Kang, S. G., Hah, C., and Jang, J. C. (2015). The tandem CCCH zinc finger motif is required for *Arabidopsis thaliana* TZF1 protein localization to cytoplasmic foci. ***Submitted*****.**

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

Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:

More producers are achieving greater yields from crop management decisions based on the quantification of the roles of genetics, population, fertilizer, foliar protection, and other inputs for maize and soybean yield and biomass production from photosynthate utilization. Across all corn hybrids, marked yield improvements by as much as 22 bu Ac^{-1} occurred due to season-long fertigation of N, K, and S along with higher populations. Nutrients (N, K, and S) were also fertigated in soybean and improved grain yield by as much as 6 bu Ac^{-1}. These findings highlight significant corn and soybean yield increases associated with in-season nutrient fertigation, and how agronomic management and cultivar selection can be used to complement improvements in nutrient recovery. Additionally, banding P fertilizer within 3 inches of the crop row produces greater yield than broadcast applications.

Accomplishments:

1. Across all corn hybrids, marked yield improvements by as much as 20 to 22 bu Ac^{-1} occurred due to season-long fertigation of N, K, and S along with higher populations.
2. Nutrients (N, K, and S) were also fertigated in soybean and improved grain yield by as much as 6.1 bu Ac^{-1}.
3. Banded P fertilizer within close proximity to the corn plant has several advantages over broadcast applications, enabling plants to better use the fertilizer during the critical stages of early growth when the yield potential is being established. Broadcast P fertilizer applications may be warranted when planting operations cannot match the fertilizer bands within 3 inches of the crop row.
4. Preliminary results show that a perennial cover crop of creeping red bentgrass can be used in combination with minimal nitrogen inputs to grow the high biomass tropical maize crop in a sustainable system.

Plans for Coming Year:

- Evaluate fertigation of nutrients to increase availability, recovery, and yield in corn and soybean production systems
- Assess the value of mechanical and chemical residue management and other agronomic inputs in continuous corn production systems.
- Evaluate crop management aspect decisions on yield of corn and soybean.

Publications (2015):

Ruffo, M.L., Gentry, L.F., Henninger, A.S., Seebauer, J.R., and Below, F.E. (2015). Evaluating management factor contributions to reduce corn yield gaps. Agronomy Journal 107:495-505.

Bender, R.R., Haegele, J.W., and Below, F.E. (2015). Nutrient uptake, partitioning, and remobilization in modern soybean varieties. Agronomy Journal 107:563-573.

Bender, R.R., Haegele, J.W., and Below, F.E. (2015). Modern soybean varieties' nutrient uptake patterns. Better Crops with Plant Food 99(2):7-10.

Kansas Agricultural Experiment Station Report

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Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:

We have improved our understanding of high temperature stress in wheat and grain sorghum. Warm nights (24 C) can be just as damaging to wheat as hot daytime conditions (35 C)—particularly during floral development. Sorghum seed set was most sensitive to hot temperatures during the period from 10 days before flowering through five days after flowering; high temperatures also reduced individual grain weight. A water-conserving trait in sorghum is expressed less frequently under hot conditions (37 C) than warm conditions (31 C). Carbon isotope (13C) discrimination, measured in wheat grain developed under post-flowering drought, can help identify germplasm with drought stress avoidance.

Accomplishments:

1. We compared the effects of high daytime and high night-time temperatures during anthesis on physiological (chlorophyll fluorescence, chlorophyll concentration, leaf level photosynthesis, and membrane damage), biochemical (reactive oxygen species (ROS) concentration and antioxidant capacity in leaves), growth and yield traits of wheat genotypes (Ventnor and Karl 92). Plants were grown at optimum temperatures (25/15 C, maximum/minimum) until the onset of anthesis. Thereafter, plants were exposed to high night-time (HN, 25/24 C), high daytime (HD, 35/15°C), high daytime and night-time (HDN, 35/24 C) or optimum temperatures for 7 days. Compared with optimum temperature, HN, HD and HDN increased ROS concentration and membrane damage and decreased antioxidant capacity, photochemical efficiency, leaf level photosynthesis, seed set, grain number and grain yield per spike. Impact of HN and HD was similar on all traits. Greater impact on seed set, grain number and grain yield per spike was observed at HDN compared with HN and HD. These results suggest that HN and HD during anthesis cause damage of a similar magnitude to winter wheat.

2. We identified the most sensitive stages of reproductive development to high temperature stress and quantified the thresholds for temperature and duration for floret fertility and individual grain weigh in sorghum. Periods between 10 and 5 d before anthesis; and between 5 d before- and 5 d after-anthesis were most sensitive to high temperatures causing maximum decreases in floret fertility. Mean daily temperatures >25°C quadratically decreased floret fertility (reaching 0% at 37°C) when imposed at the start of panicle emergence. Temperatures ranging from 25 to 37°C quadratically decreased individual grain weight when imposed at the start of grain filling. Both floret fertility and individual grain weights decreased quadratically with increasing duration (0–35 d or 49 d during floret development or grain filling stage, respectively) of high

temperature stress. In field conditions, imposition of temperature stress (using heat tents) during floret development or grain filling stage also decreased floret fertility, individual grain weight, and grain weight per panicle.

3. A limited-transpiration (TRlim) trait has been identified in many crop species, including sorghum (Sorghum bicolor (L.) Moench), that results in restricted transpiration rate under high vapor pressure deficits (VPD). The benefit of TRlim is that under high midday-VPD conditions crop water loss is limited so that there is water conservation and positions the crop to better withstand later-season drought. Previous studies performed at 31°C found that TRlim was commonly expressed among sorghum genotypes. However, it is uncertain how applicable these previous results obtained at 31°C might be at higher temperature that may exist at midday in regions where sorghum is commonly grown. The current study tested for the expression of TRlim at 37°C in 16 sorghum genotypes previously found to express the trait at the lower temperature. Only three of the genotypes sustained expression of TRlim at 37°C. These results indicate that for environments where temperature may commonly reach or exceed 37°C, sorghum genotypes have been favored that acclimate to the high temperature by losing the TRlim trait. In conditions in which very high temperatures threaten crop heat stress, those genotypes that lose the TRlim trait at high temperature may be more desirable since increasing transpiration rates at these temperatures can result in leaf cooling.

4. We found that wheat grown in water-deficient intensive cropping systems had less biomass productivity relative to crop water use. This finding contrasted with evidence of greater transpiration efficiency for wheat in intensive systems, indicated by smaller grain carbon isotope (13C) discrimination (CID). The reduced water productivity at the cropping system level was attributed to a smaller transpiration fraction of crop water use for wheat in the more intensive cropping systems.

5. We examined the genetic variation of grain carbon isotope discrimination (CID), an indicator of crop water productivity. Field studies indicated that both preliminary and advanced breeding lines had significant genetic variations and relatively small coefficients of variation in grain CID, indicating grain CID is a promising trait for selection in wheat breeding programs. In the trials for the preliminary breeding lines, the association between grain CID and grain yield was significant and positive in three environments. This correlation was stronger in a trial with expected post-anthesis drought stress. However, there was only a weak positive correlation between grain CID and grain yield in the trial for advanced breeding lines. Winter injury may have confounded the grain CID and yield relationship. This study also revealed a significant and negative correlation between grain CID and grain protein content in all four trials, suggesting a possible impact on baking quality while selecting for high grain CID. Therefore, our results suggest that grain CID could be useful for grain yield prediction in semi-arid areas with moderate drought stress. However, precaution should be taken for selecting grain CID because of the effect of environment on its association with grain yield and its negative correlation with protein content.

Plans for Coming Year (2016):

- Evaluate impact of high temperature stress on reproductive processes and yield formation in grain crops.

- Understand mechanisms associated with tolerance for high temperature and drought tolerance.
- Develop and apply novel phenotypic methods, including canopy imaging techniques, to screen germplasm for tolerance to high temperature and drought stress in controlled environments in laboratory and field conditions.

Publications (2015):

Godar, A.S., Varanasi, V.K., Betha, S., Prasad, P.V.V., Thompson, C.R., and Mithila, J. 2015. Physiological and molecular mechanisms of differential sensitivity of palmer amaranth (*Amaranthus palmeri*) to mesotrione at varying growth temperatures. PloS One 10(5), e0126731.

Narayanan, S., Prasad, P.V.V., Fritz, A.K., Boyle, D.L., and Gill, B.S. 2015. Impact of high nighttime and high daytime temperature stress on winter wheat. Journal of Agronomy and Crop Science 201, 206-218.

Pradhan, G.P., and Prasad, P.V.V. 2015. Evaluation of wheat chromosome translocation lines for high temperature stress tolerance at grain filling stage. PLoS One 10(2), e0116620.

Prasad, P.V.V., Djanaguiraman, M., Perumal, R., and Ciampitti, I.A. 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Frontiers in Plant Science 6,820.

Riar, M.K., Sinclair, T.R., and Prasad, P.V.V. 2015. Persistence of limited-transpiration-rate trait in sorghum at high temperature. Environmental and Experimental Botany 115, 58-62.

Zhang, G., R. Aiken, T.J. Martin. 2015. Relationship between Carbon Isotope Discrimination and Grain Yield of Rainfed Winter Wheat in a Semi-arid Region. Euphytica 204:1 39-48 doi:10.1007/s10681-014-1335-6

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Objective 4: Developmental and Environmental Limitations to Photosynthesis

Impact Statement:
A thorough understanding of soybean photosynthetic responses to high temperatures and underlying regulatory mechanisms is critical to identify and efficiently improve performance under non-optimal temperature conditions. We have identified soybean genotypes that can be employed for genetic analysis of heat-tolerance mechanisms and used in breeding programs to improve soybean heat tolerance. In addition, the mechanistic understanding gained is expected to reveal approaches that can be leveraged to improve soybean heat tolerance. In the long term, soybean farmers will benefit from the development of more heat tolerant soybean cultivars.

Accomplishments:
1. Characterized 120 soybean genotypes under high temperature conditions in the greenhouse for chlorophyll fluorescence
2. Identified extreme soybean phenotypes under high-temperature conditions in the greenhouse and characterized non-photochemical quenching and chlorophyll fluorescence traits under ambient and high temperature conditions in the field.
3. Determined non-structural carbohydrates in leaves of field-grown soybean genotypes in response to ambient and high temperature conditions.
4. Employed genome wide association analyses to identify genomic regions associated with soybean leaf and canopy characteristics including carotenoid content, chlorophyll content, and photochemical reflectance index.

Plans for Coming Year:
- Continue physiological characterization of contrasting soybean genotypes under ambient and high temperature conditions.
- Characterize the relationship of leaf tissue carbohydrate accumulation under heat stress and chlorophyll fluorescence responses.
- Collaborate with T. Sharkey on the examination of transgenic soybean for isoprene emission.

Publications (2015):
Dhanapal, A.P., J.D. Ray, S. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, and F.B. Fritschi. 2015. Association mapping of total leaf carotenoids in diverse soybean [*Glycine max* (L.) Merr.] genotypes based on leaf extracts and canopy spectral reflectance. PLOS ONE.

Houx, J.H., III., and F.B. Fritschi. 2015. Influence of late planting on light interception, radiation use efficiency and biomass production of four sweet sorghum cultivars. Industrial Crops and Products.

Dhanapal, A., J.D. Ray, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, P.B. Cregan, Q. Song, F.B. Fritschi. 2015. Genome-wide association study (GWAS) of carbon isotope ratio ($\delta^{13}C$) in diverse soybean [*Glycine max* (L.) Merr.] genotypes. Theoretical and Applied Genetics 128:73-91.