**NC1200 – Regulation of Photosynthetic Processes**

**2016 Annual Meeting**

Location: Montana State University, Bozeman, MT

Room: 108 PBB

<http://calendar.msu.montana.edu/locations.php?building=35>

**Friday, September 30, 2016**

18:15 Meet in hotel lobby, travel to Montana Ale Works (Main St.) for 18:30 Dinner

**Saturday, October 1, 2016**

Breakfast on your own but there will be snacks in the meeting room.

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

8:00 Meet in hotel lobby to travel to campus

8:30 *Michael Giroux* – Montana State University

9:00 *Tasios Melis* – University of California, Berkeley

9:30 *Jeffrey Harper* – University of Nevada, Reno

10:00 Break

10:30 *Steve Huber* – University of Illinois

11:00 *Glenda Gillaspy* – Virginia Tech

11:30 *Rebecca Roston* – University of Nebraska

12:15 Lunch – Bridger Brewing (meal and alcohol-free drinks included in registration)

13:30 *Krishna Jagadish* – Kansas State University

14:00 *Thomas Sharkey* – Michigan State University

14:30 *Andreas Fischer* – Montana State University

15:00 Break

15:30 *Asaph Cousins* - Washington State University

16:00 General discussions and business meeting

18:00 Meet in hotel lobby, travel to Open Range (Main St.) for 18:15 dinner

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## Doug K. Allen, Missouri AES/ARS

**Objective 3: Mechanisms Regulating Photosynthate Partitioning**

**Impact Statement:** The dynamics of metabolism are a consequence of cellular regulation and reflect changes in the environment. Methods that can better depict and quantitatively assess metabolism including both source and sink, provide a description of network operation within the plant that can guide metabolic engineering efforts to enhance crop productivity.

**Accomplishments:**

1. Lipid Metabolism: Lipids are the most highly reduced form of carbon storage, yet our understanding of lipid metabolism is incomplete. Current methods to analyze the dynamics of lipid metabolism are largely predicated on radiolabeling experiments; however mass spectrometry is highly informative about labeling within individual carbon atoms of a molecule. Mass spectrometry of lipids can be particularly difficult for a multitude of reasons which have limited attempts to label and analyze lipids with stable isotopes. We have developed initial methods that start to address some of these challenges by using high resolution mass spectrometry. The approach is under further refinement but could potentially offer significant insight over traditional methods to examine the lipid metabolic network.

2. C4 Photosynthesis: In collaboration with Tom Brutnell’s lab we investigated a malate transporter that strongly impacts the growth phenotype of maize. A combination of biochemical studies, microscopy, and isotopic labeling with both radio- and stable isotopes were used to quantify metabolism and gain insight to its operation. The results emphasized that multiple C4 sub-pathways involving PEPC and NADP-ME are important to photosynthesis and may possibly partially compensate for one another when plants are genetically altered.

**Plans for Coming Year:**

* Refine methods for lipid analysis by mass spectrometry. Though we have developed a proof of concept, considerable work remains to be done to quantify the labeling in different lipids.
* Complete metabolic flux analysis and physiological characterization of an Arabidopsis line overexpressing carbonic anhydrase.

**Publications (2016):**

**I Couso, B Evans, J Li, Y Liu, F Ma, G Anderson, S Diamond, DK Allen, JG Umen** (2016).Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control and lipid metabolism in Chlamydomonas. *Plant Cell (accepted).*

**M Hodges, Y Dellero, O Keech, M Betti, AS Raghavendra, R Sage, X Zhu, DK Allen, APM Weber** (2016). Perspectives for a better understanding of the metabolic integration of photorespiration within a complex plant primary metabolism network. *Journal of Experimental Botany 67:3015-3026*.

**DK Allen** (2016). Assessing Compartmentalized Flux in Lipid Metabolism with Isotopes. *Biochimica Biophysica Acta – Molecular & Cell Biology of Lipids* 1861:1226-1242.

**S Weissmann, F Ma, K Furuyama, JK Gierse, H Berg, Y Shao, M Taniguchi, DK Allen, TP Brutnell** (2016). Interactions of C4 subtype metabolic activities and transport in maize are revealed through the characterization of dct2 mutants. *Plant Cell* 28:466-484.

**DK Allen** (2016). Quantifying plant phenotypes with metabolic flux and isotopic labelling. *Current Opinion in Biotechnology* 37:45-52.

## Fred E. Below, 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. Increasing corn yield using greater plant populations needs concurrent placement in narrower rows for greatest solar and nutrient use. Growing corn continuously typically results in reduced yield compared to a corn- soybean rotation. However, the majority of the continuous corn yield penalty can be mitigated by using enhanced fertility and fungicidal leaf protection, combined with residue management and hybrid selection. Significant corn and soybean yield increases associated with in-season nutrient fertigation, in combination with agronomic management and cultivar selection can be attributed to improvements in photosynthesis.

**Accomplishments:**

1. Yield increases by as much as 50 bushels/ acre occurred in corn, and 10 bushels/ acre for soybean were produced by timely fertigation of N, P, K, S, and Zn along with higher populations.
2. Corn grown in the 13th continuously cropped year yields, on average 48 bushels/ acre less than when rotated with soybean. However, using sidedressed N, slow- release P, K, and S, and a fungicide with 45,000 plants per acre brought continuous corn yields, on average, within 3 bushels/ acre of rotated yields.
3. In continuous corn, residue degradation by mechanical or chemical means improved corn emergence and yield.
4. While corn yield decreases, on average, when increasing corn plant population from 38 to 44 thousand plants/ acre; allowing the individual plants more room in 20” rows compared to conventional 30” rows increases yield by 9 bushels/ acre at the greater population.

**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 decisions on yield of corn and soybean.

**Publications (2016):**

**Below, F.E.,** **Beyrer, T.A., Mastrodomenico, A.T., and Seebauer, J.S.** (2016). Efficient management of water and nutrient resources: Assessing the potential for drip irrigation and fertigation. Proceedings of the 2016 Fluid Fertilizer Forum. 15-16 February, Scottsdale, AZ.

## 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 and published. Notably, the viability of the mutant was reduced, showing the importance of CrTGD2. Galactoglycerolipid metabolism was altered in the tgd2 mutant with monogalactosyldiacylglycerol (MGDG) synthase activity being strongly stimulated. We hypothesize this to be a result of phosphatidic acid accumulation in the chloroplast outer envelope membrane, the location of MGDG synthase in Chlamydomonas. Concomitantly, increased conversion of MGDG into triacylglycerol (TAG) was observed. This TAG accumulated in lipid droplets in the tgd2 mutant under normal growth conditions. Labeling kinetics indicate that Chlamydomonas can import lipid precursors from the ER, a process that is impaired in the tgd2 mutant.
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. To follow up on this observation, we generated chimera of the Arabidopsis and Brachypodium TGD1 protein and introduced them into the Arabidopsis *tgd1* mutant for complementation. We identified a specific loop of TGD1 that is required for complementation. Protein modeling suggests that this loop interacts with another component of the TGD123 lipid transport complex. This hypothesis is currently tested.
3. We collaborated in the analysis of a ferredoxin-5 mutant of Chlamydomonas leading to altered lipid metabolism and thylakoid disruption in the dark.

Under Objective 3:

1. The detailed phenotypic analysis of the cht7 mutant cells of Chlamydomonas has been nearly completed. We showed that cell cycle genes are misregulated during N-deprivation. This is likely causal to other morphological cell phenotypes observed during N deprivation and N-refeeding. We have also begun to study the cht7 mutant and the wild type in photobioreactors. We established conditions to synchronize the growth of the cells, which will allow us to compare the transcriptome of mutant and wild type at different stages of the cell cycle. In addition, we developed new cell lines combining cell cycle mutations and the cht7 mutation into compatible background for further study.
2. An extensive analysis of diacylglycerol acyltransferase involved in triacylglycerol formation in Nannochloropsis was completed. A specific isoform has been explored for its efficacy in lipid droplet formation and enhancement of energy density of leaves in transgenic plants.
3. Cocultivating the alga Nannochloropsis and an oleogenic fungus Mortierella, we observed synergistic enhancement of oil production. Moreover, we discovered that the algal cells enter the fungal hyphae and are viable and dividing. In essence we were able to observe the initial steps of an endosymbiotic event reproducibly in the test tube. Because both organisms are experimentally tractable at the molecular level, this new experimental system provides an opportunity to gain mechanistic insights into the establishment of an endosymbiotic interaction between a photosynthetic alga and a heterotrophic fungus.
4. The ectopic expression of WRI1 in Brachypodium led to increased lipid droplet formation in leaf blades but also induced localized cell death. To overcome this problem construct were tested expressing WRI1 and other genes from algae under the control of stem-specific promoters. The analysis of these plants is ongoing.
5. The interaction of WRI1 with other transcription factors is investigated. The focus is currently on two specific transcription factors to determine their mode of interaction with WRI1.

Under Objective 4:

1. A study of the recombinant SFR2 protein of tomato has been completed. RNAi repression of SFR2 in tomato led to increased salt and drought sensitivity of tomato. In Arabidopsis, loss of SFR2 led to freezing sensitivity but not to sensitivity to salt or drought stress distinguishing the function of SFR2 in freezing sensitive and resistant plants.
2. The analysis of three chloroplast located lipases of Arabidopsis has been nearly completed. Overexpression of these proteins led to changes in thylakoid membrane lipid profiles that suggest that in vivo they prefer particular molecular species of phosphatidylglycerol or monogalactosyldiacylglycerol. Based on pulse chase experiments and lipid analysis of mutant and wild-type developing seeds of Arabidopsis we hypothesize that these lipases are involved in acyl exchange and participate in the export of fatty acids from the chloroplast in developing embryos.
3. The analysis of photosynthetic alterations in the low triacylglycerol mutant of Chlamydomonas, *pdgl1¸* is nearly complete. The mutant showed altered chlorophyll fluorescence and composition of the photosynthetic apparatus as well as increased light sensitivity leading to reactive oxygen formation.

**Plans for Coming Year:**

1. The analysis of TGD1 proteins in Brachypodium and Arabidopsis will be completed as will be the analysis of recombinant PGDL proteins. The role of PGDL lipases in seed oil biosynthesis will be further explored. Moreover, we are planning to gain a mechanistic understanding of growth inhibition in PGDL overproducing lines. We have first indications that these lipases release acyl groups from thylakoid membrane lipids and that these acyl groups serve as precursors for jasmonate biosynthesis.
2. A major effort will continue to be on the identification of direct target genes of the CHT7 complex in Chlamydomonas. The in-depth analysis of the *cht7* mutant phenotype will be completed. The physiological analysis of the Chlamydomonas *pgd1* mutant will be completed and published.
3. We will complete the initial analysis of the newly discovered interaction between Nannochloropsis and Mortierella and publish a first paper on this new system. Subsequent studies will be pursued to explore whether we can gain mechanistic insights into the initial steps of an endosymbiotic event.

**Publications (2015/2016 since last report):**

1. Yang, W., T. M. Wittkopp, X. Li, W. Warakanont, A. Dubini, C. Catalanotti, R. G. Kim, E. C. M. Nowack, L. C. M. Mackinder, M. Aksoy, M. D. Page, S. D’Adamo, S. Saroussi, M. Heinnickel, X. Johnson, P. Richaud, J. Alric, M. Boehm, M. C. Jonikas, C. Benning, S. S. Merchant, M. C. Posewitz, and A. R. Grossman. 2015. Critical role of *Chlamydomonas reinhardtii* ferredoxin-5 in maintaining membrane structure and dark metabolism Proc. Natl. Acad. Sci. USA 112:14978-83.
2. Handee,W., Li, X., Hall, K.W., Deng, X., Li, P., Benning, C., Williams, B.L., Kuo, M.H. 2016. [An Energy-Independent Pro-longevity Function of Triacylglycerol in Yeast](http://www.ncbi.nlm.nih.gov/pubmed/26907989). PLoS Genet. 12:e1005878. doi: 10.1371/journal.pgen.1005878.
3. Barnes, A.C., Benning, C., Roston, R. 2016. [Chloroplast membrane remodeling during freezing stress is accompanied by cytoplasmic acidification activating SENSITIVE TO FREEZING2](http://www.ncbi.nlm.nih.gov/pubmed/27233750). Plant Physiol. 171:2140-9. doi: 10.1104/pp.16.00286.
4. Ma, W., Kong, Q., Mantyla, J.J., Yang, Y., Ohlrogge, J.B., Benning, C. 2016. 14-3-3 protein mediates plant seed oil biosynthesis through interaction with AtWRI1. Plant J. doi: 10.1111/tpj.13244. (Epub ahead of print).
5. Wang, K., Hersh, H.L., Benning, C. 2016. [SENSITIVE TO FREEZING2 aides in resilience to salt and drought in freezing-sensitive tomato](http://www.ncbi.nlm.nih.gov/pubmed/27600812). Plant Physiol. pii: pp.01183.2016 (Epub ahead of print).
6. Kelly, A.A., Kalisch, B., Hölzl, G., Schulze, S., Thiele, J., Melzer, M., Roston, R.L., Benning, C., Dörmann, P. 2016. Proc. Natl. Acad. Sci U S A pii: 201609184. (Epub ahead of print).
7. Zienkiewicz K, Du Z.-Y., Ma W., Vollheyde K., Benning C. (2016). Stress-induced neutral lipid biosynthesis in microalgae - Molecular, cellular and physiological insights. Biochim. Biophys. Acta. 1861:1269-81. doi: 10.1016/j.bbalip.2016.02.008.
8. Horn, P.J., Benning, C. 2016. The plant lipidome in human and environmental health. Science 353: 1228-32. doi: 10.1126/science.aaf6206

## Asaph Cousins, WA-AES

**Objective #: Photosynthetic Capture and Photorespiratory Release of CO2**

**Impact Statement:**

The measurements of leaf carbon and oxygen stable isotopes were tested to estimate water use efficiency in a model C4 grass system.

**Accomplishments:**

1. We are screening a population of recombinant inbreed lines (RILs) of *Setaria viridis* and *Setaria italica* grown under field conditions at the University of Illinois for variation in water use efficiency using carbon and oxygen stable isotopes (δ13C and δ18O, respectively). We have collected samples from 2013, 2014, 2015 and 2016 for both a density and drought experiment and we have analyzed 839 and 757 samples for drought and density experiments, respectively. Additionally, we have analyzed 358 RIL and 507 *S. viridis* accession samples in a drought experiment conducted by the Baxter lab in the Danforth phenotyper. QTLs that contribute to variation in foliar δ13C and δ18O have been mapped to compare QTLs under high/low density and well-watered/drought conditions.

2. A manuscript for a controlled environment drought experiment conducted on the parental lines A-10 (*S. viridis*) and B-100 (*S. italica*) is currently being prepared. Another manuscript testing thermography and spectral properties of leaves to measure transpiration rate and water use efficiency has been published.

**Plans for Coming Year:**

* Write and submit manuscripts on 13C and 18O variation across RILs and associated QTLs in response to drought and density experiments
* Submit manuscript on controlled environment drought experiment on parental lines A-10 and B-100.

**Publications (2015 and 2016):**

**Ellsworth P.Z., Cousins A.B.** (2016) Carbon isotopes and water use efficiency in C4 plants. Current Opinion in Plant Biology 31, 155–161

**Wang M., Ellsworth P.Z., Zhou J., Cousins A.B. Sankaran S.** (2015) Evaluation of water-use efficiency in foxtail millet (Setaria italica using visible-near infrared and thermal spectral sensing techniques. Talanta 152, 531–539

## John C. Cushman, Nevada AES

**Objective 4: Developmental and Environmental Limitations to Photosynthesis.**

**Impact statement(s):**

The long-term goal of our research is to improve water-use efficiency (WUE) in plants. WUE is defined as the ratio of carbon assimilation resulting in biomass accumulation to the rate of water lost due to transpiration. Improving WUE is a desirable goal because it would allow plants to be grown with less water. This will become an increasing important goal in the context of global climate change as agricultural water resources become limiting particularly in more arid regions. Our research efforts have focused on a specialized mode of photosynthesis called crassulacean acid metabolism (CAM), which features nocturnal CO2 uptake, which promotes increased WUE, and enables plants to inhabit water-limited environments. Current research is focused on characterizing the genome-scale requirements of CAM using comparative genomics approaches using model and agronomically important CAM species, exploring the anatomical benefits of tissue succulence, a trait this is often associated with CAM, and using bioengineering approaches to transfer the CAM pathway into C3 photosynthesis species.

**Accomplishments:**

1. Completed the sequencing of a high-quality transcriptome including miRNAs and genome of the common ice plant (*Mesembryanthemum crystallinum*), a facultative CAM species.
2. Characterized candidate genes responsible for the formation of thick leaves and epidermal bladder cells in *Mesembryanthemum crystallinum.*
3. Successfully engineered tissue succulence in *Arabidopsis thaliana* resulting in improved biomass, seed production, WUE, drought attenuation, and salinity tolerance.
4. Made significant progress in obtaining a comprehensive transcriptome for prickly pear cactus (*Opuntia ficus-indica*), an agronomically important obligate CAM species,using Pac Bio Iso-Seq full-length transcript sequencing.
5. Continued work on a field study in Logandale, NV to examine the biomass production of three *Opuntia* species under three different irrigation regimes and initiated a new field trial of cold-tolerant *Opuntia* hybrids.
6. Made significant progress towards completing the genome sequence of the prickly pear cactus (*Opuntia cochenillifera*), a diploid, obligate CAM reference species.
7. Completed the construction of synthetic gene circuits for the basic carboxylation and decarboxylation modules of CAM for introduction into *Arabidopsis thaliana.*

**Plans for the Coming Year:**

* Complete the salinity and drought stress tolerance assessment of *Arabidopsis thaliana* with engineered tissue succulence.
* Publish the results of our analysis of the *Mesembryanthemum crystallinum* transcriptome and genome.
* Complete the analysis of the transcriptome of the *Opuntia ficus-indica*, an octoploid, obligate CAM species.
* Complete the transcriptome and genome sequencing of the *Opuntia cochenillifera*, a diploid, obligate CAM reference species.
* Complete the introduction of a variety of synthetic gene circuits of the basic carboxylation and decarboxylation modules of CAM into *Arabidopsis thaliana* and complete the molecular and physiological analysis of selected transgenic lines. Work towards moving these same CAM modules into *Populus*, a SRF bioenergy crop.
* Complete the detailed molecular analysis of a thick-leaf mutant and an epidermal bladder cell-less mutant of the common ice plant *(Mesembryanthemum crystallinum* L.).

**Publications (2016):**

**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**, 20. http://dx.doi.org/10.3389/fenrg.2015.00020

**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. 47: 1435–1442. DOI:10.1038/ng.3435.

**Borland, A.M., Guo, H-B., Yang, X., Cushman, J.C.** (2016) Orchestration of carbohydrate processing for crassulacean acid metabolism. Current Opinion in Plant Biology. 31: 118–124.

**Chiang, C-P., Yim, W.C., Sun, Y-H., Ohnishi, M., Mimura, T., Cushman,J.C., Yen,H.E**. (2016) Identification of ice plant (*Mesembryanthemum crystallinum* L.) microRNAs using RNA-Seq and analysis of their potential functional roles in high salinity responses in seedlings. Frontiers in Plant Biology. 7: 1143. DOI: 10.3389/fpls.2016.01143.

**Roeurn, S., Hoshino, N., Soejima, K., Inoue, Y., Cushman, J.C., Agarie, S**. (2016) Suppression subtractive hybridization library construction and identification of epidermal bladder cell-related genes in the common ice plant, *Mesembryanthemum crystallinum* L. Plant Production Science. *In press.* DOI:10.1080/1343943x.2016.1221320.

**Abraham, P.E., Yin, H., Borland, A.M., Weighill, D., Lim, S.D., De Paoli, H.C., Engle, N., Agh, R., Weston, D.J., Wullschleger, S.D., Tschaplinski, T., Jacobson, D., Cushman, J.C., Hettich, R.L., Tuskan, G.A., Yang, X.** (2016) The temporal dynamics of differential transcriptional and translational behaviors and the metabolic processes that define crassulacean acid metabolism in *Agave*. Nature Plants. *In press.*

**Roeurn, S., Hoshino, N., Soejima, K., Inoue, Y., Cushman, J.C., Agarie, S.** (2016) HD-ZIP IV and MYB homologs related to trichome formation are involved in epidermal bladder cell development in the halophyte *Mesembryanthemum crystallinum* L. Biologia Plantarum. *In press.*

**Other:** *Optional (e.g.*, patents, awards, etc.)

**Patents:**

**Lim, S.D. and Cushman, J.C.** (2015) Methods of Engineered Tissue Succulence in Plants. Provisional US Patent #62255158, Filed Nov. 13, 2015.

**Lim, S.D., Yim, W.C. and Cushman, J.C.** (2016) Methods of Engineered Crassulacean Acid Metabolism (CAM) in Plants. Provisional US Patent #62276438, Filed Jan. 08, 2016.

**Collaborations:**

A collaboration was initiated with Jeff Harper (NC1200 member) to improve water-use efficiency and yield in *Camelina sativa* (cv. ‘Celine’).

## 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 inositol phosphate signaling molecules including InsP6, InsP7, and InsP8. We have previously examined plant inositol kinase genes and the role they play in driving InsP6, InsP7, and InsP8 synthesis in plants, and how this relates to energy sensing. Our second target for controlling energy sensing in plants is the sucrose non-fermenting related kinases (SnRKs), which are major energy sensors in eukaryotes that impact metabolism, global transcription programs, stress signaling and lifespan. Previous work has shown that overexpression of the SnRK1 gene can increase biomass production in late development, and might be a useful tool to increase plant productivity.

**Accomplishments:**

1. Completed metabolic analyses of inositol kinase and SnRK1 mutant plants.
2. Developed initial version of a genome scale and “transitional” model to understand SnRK1 impacts on metabolism.
3. Established a pistil-drip cotton transformation system to produce cotton transgenics altered in IPK1 and SnRK1 genes.

**Plans for Coming Year:**

* Refine observed changes in inositol phosphates and energy status.
* Investigate new enzymes responsible for InsP7 and InsP8 breakdown.
* Refine transitional genome scale model.
* Characterize cotton transgenic plants altered n SnRK1.

**Publications (2015-2016):**

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.

## Mike Giroux, Department of Plant Sciences Montana State University

## 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.

**2016 Accomplishments:**

1. Completed gene expression experiments and found genes involved in carbon metabolism to be generally up-regulated in response to increased AGPase expression in leaves, seeds, or both.
2. Determined that carbon metabolites were broadly up-regulated in response to increased AGPase expression in leaves as well as seeds with an additive up-regulation in plants with overexpression of AGPase in both leaves and seeds. This is interesting in that it indicates that the yield advances observed in the yield trial were achieved via broad-upregulation of carbon metabolism and did not involve changes in photosynthesis on a per unit basis.
3. Summarized results and submitted to be published.
4. Began examining these genotypes when grown under variable Nitrogen concentrations.

**Plans for Coming Year:**

* Examine starch concentrations in leaves and seeds collected at the end of the photoperiod for wild type (WT) and plants with overexpression AGPase in both leaves and seeds (LS) under 5 nitrogen concentrations. Tissue has been collected at one month after planting, anthesis, 14 DAF and maturity.
* Complete replicated yield trials for WT and LS genotypes grown under 50 ppm, 75 ppm, 100 ppm, 150 ppm and 200 ppm N regimes.

**Publications:**

**2016**

Oiestad A.J., Martin J.M., Giroux M.J. (in press) Overexpression of ADP-glucose pyrophosphorylase in both leaf and seed tissue synergistically increase biomass and seed number in rice (*Oryza sativa* L. ssp. japonica). <http://dx.doi.org/10.1071/FP16218>

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

**Objective 3: Partitioning of Photosynthate**

**Objective 4: Developmental and environmental limitations to photosynthesis.**

**Impact Statement: *The long-term goal*** is to make crop plants more tolerant to abiotic stresses, such as heat, cold, drought and salt. A specific area of focus is on heat stress and sexual reproduction in flowering plants. 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 highly sensitive 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 investigates the structure and biological functions of genes ecoding 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 stimulus-specific Ca2+ signals are used to coordinate the activity of multiple cellular processes. A specific focus is on how calcium-dependent protein kinases (CPKs) can regulate processes important for cellular growth, such as turgor pressure, the secretion of new cell wall materials, and feedback signaling between cell-wall structures and the growth machinery within the cytoplast.

**Accomplishments:**

1. We have obtained genetic evidence that autophosphorylation of a specific tyrosine in CPK34 is important for regulating cellular processes important to pollen fertility. Previous biochemical evidence indicated that tyrosine autophosphorylation by a CPK can attenuate its kinase activity (as discovered by Steve Huber (NC1200 member) et al., in 2012 (*FEBS Lett* *586*, 4070-4075)). To examine the *in planta* function of this auto-inactivation mechanism, we compared the phenotypes of pollen expressing CPK34 transgenes engineered with either a phospho-block substitution (Y to F, i.e., cannot autoinactivate) or phospho-mimic (Y to E, i.e., always inactivated). While pollen transmission efficiencies were normal for pollen expressing a CPK34 phospho-mimic (CPK34-YtoE), the Y to F phospho-block substitution increased pollen transmission efficiencies by more than 2-fold (when assayed in competition with wild type pollen). This improvement in pollen fitness provides the first example of a biological function associated with of a CPK-dependent phospho-tyrosine activity
2. A collaboration was initiated with John Cushman (NC1200 member) to improve water use efficiency and yield in *Camelina sativa* (cv. ‘Celine’). Recently, the Cushman lab successfully engineered tissue succulence in *A. thaliana* through the overexpression of a “CEB1” transcription factor associated with grape berry development. These CEB-modified plants show greater water-retention capacity and improved drought tolerance. Preliminary results in the Harper lab indicate similar plant growth and yield enhancements in *Camelina*.

**Plans for Coming Year:**

* Test candidate stress-tolerance genes for their ability to improve heat-stress tolerance in pollen.
* Test whether CPKs with phospho-tyrosine related changes can be used to enhance growth or heat stress tolerance in pollen and other plant cells.
* 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 (2016):**

**Eaves, DJ., T Haque, NPJ Cotton, CG Zampronio, RL Tudor, Y Baron, HJ Cooper, SA White, CH Franklin, JF Harper, VE. Franklin-Tong** (2016). Identification of Phosphorylation Sites that Alter Activity of Pollen Soluble Inorganic Pyrophosphatases. Plant Phys. (submitted).

## 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. Plants contain a unique family of ‘sensor responders’ that are calcium-dependent protein kinases (CPKs) that are activated by calcium binding and link changes in calcium with alterations in protein phosphorylation. While regulation of CPKs by calcium has been well studied, the potential contribution of other factors such as autophosphorylation of the CPK and possible interaction with sensor relay proteins has not been fully explored. Studies with one member of the Arabidopsis CPK family, CPK28, has documented autophosphorylation on numerous serine, threonine and tyrosine residues, and secondly that CPK28 interacts in a high-affinity manner with the ‘sensor relay’ protein, calcium/calmodulin. Interestingly, the autophosphorylation status of CPK28 reduces the interaction with calcium-bound calmodulin, and eliminates the dependence of kinase activity on added calcium. The latter result indicates that when autophosphorylated, CPK28 directly binds calcium with increased affinity such that basal levels of calcium are sufficient to maintain full activity. This suggests a molecular mechanism to allow the protein kinase to ‘remember’ prior calcium-signaling events. Overall, the results uncover new complexities in calcium signaling in plants.

2. Two of the most studied plant receptor kinases are BRI1 and BAK1, which function together in brassinosteroid signaling. Activation of the BRI1 and BAK1 protein kinase domains is known to involve phosphorylation but other mechanisms have not been investigated. Therefore, we conducted molecular dynamic simulations of the phosphorylated, active protein kinase domains of these proteins starting with previously determined static X-ray crystal structures. The simulations revealed an unexpected tendency of the critical structural helix-C, known to be essential for eukaryotic protein kinase activity, to unfold suggesting that plant receptor kinase activity may be regulated by structural factors in addition to phosphorylation. This unexpected property may be related to regulation of activity or complex formation between the receptor and co-receptor kinases.

3. We modified the Rubisco activase protein complement in rice by eliminating the carboxy-terminal extension of the alpha form of the enzyme via genome editing. Rubisco activase is needed to activate the primary photosynthetic carbon assimilation enzyme, Rubisco, and typically it is composed of two different subunits, the alpha and beta subunits, which form a heteromeric complex involved in Rubisco activation. The modifications made (using CRIPR/Cas9-mediated genome editing) were to delete or disrupt exon 7 in the rice Rubisco activase gene, which eliminates the carboxy-end extension of one splice version of the activase protein, the so-called alpha form. The alpha form is essentially identical to the shorter beta form, except that the C-terminal extension contains two redox-sensitive cysteines responsible for inactivation of activase in the dark or low light. Our reasoning, based on previous work with Arabidopsis was that the elimination of the alpha form would result in a higher continuous activation state for Rubisco activase (would lack low-light inactivation) and thus a higher overall Rubisco activation state. The increased activation state for Rubisco should allow the plants to better take advantage of intermittent high light conditions (generally found in the lower leaves in canopies of crop plants) and thus would result in plants that would grow better in alternating low-light/high-light, mimicking canopy shading conditions in the field. These phenotypes have been observed in preliminary growth chamber experiments (collaboration with Marty Spalding, ISU).

**Plans for Coming Year:**

* In collaborative studies with M. Spalding (ISU) will analyze existing transgenic rice lines with targeted editing (truncation) of the Rubisco activase gene to further test the hypothesis that maintaining Rubisco activation state at low light enhances photosynthesis and plant growth. Of particular importance is establishing stability and heritability of the gene edits in the T2 generation; developing and analyzing a segregating population to test whether the *Rca* editing correlates with phenotype; and to obtain null segregants for CRISPR/Cas9 so that plants can be tested in the field.
* Identify in vivo interacting proteins with the receptor kinase, BRI1 in Arabidopsis, in relation to in vitro interactors that bind to a synthetic phosphopeptide corresponding to BRI1 Tyr-831. The ultimate goal is to determine the basis for regulation of BR signaling by Tyr phosphorylation of BRI1.
* Further test the regulatory role of phosphorylation of RCA Thr78 using transgenic plants and the *cpck2* knockout mutant.
* Engineer the RCA phosphosite by substituting a Ser for Thr78 (to generate the T78S directed mutant) that may have distinct properties.
* Determine whether the plastid-targeted CK2 protein kinase (cpCK2α) phosphorylates and regulates STN7 (protein kinase involved in state transitions) and whether this provides some coordination between C-assimilation and light harvesting/electron transport (collaboration with Eva-Marie Aro).

**Publications (2016):**

**Huber, S.C., Li, K., Nelson, R., Ulanov, A., DeMuro, C.M., and Baxter, I.** (2016). Canopy position has a profound effect on soybean seed composition. PeerJ 4:e2452; DOI10.7717/peerj.2452

**Kim, S.Y., Bender, K.W., Walker, B., Zielinski, R.E., Spalding, M.H., Ort, D.R., and Huber, S.C.** (2016). The plastid casein kinase 2 phosphorylates Rubisco activase at the Thr-78 site but is not essential for regulation of Rubisco activation state. Front Plant Sci., 7: 404. doi: 10.3389/fpls.2016.00404

**Soleh, M.A., Tanaka, Y., Kim, S.Y., Huber, S.C. Sakoda, K. and Shiraiwa, T.** (201X). Identification of large variation in the photosynthetic induction response among 37 soybean genotypes that is not correlated with steady-state photosynthetic capacity. Photosyn Res., in revision.

**Bender, K.W., Blackburn R.K., Monaghan, J., Zipfel, C., Goshe, M.B., Zielinski, R.E., and Huber, S.C.** (201X). Regulation of Arabidopsis thaliana Ca2+-dependent protein kinase 28 by autophosphorylation and Ca2+/Calmodulin-binding. J. Biol. Chem., in review.

**Moffett, A.S., Bender, K.W., Huber, S.C. and Shukla, D.** (201X). Conformational disorder as a potential regulatory mechanism of the BRI1 and BAK1 kinase domains in plants. Nat. Commun., in review.

**INVENTION DISCLOSURE: “**Improved Rubisco activase.” Inventors: M Spalding, D. Wright, S.Y. Kim, and S.C. Huber, Docket No. 0173.16, filed Jul 7, 2016. Provisional Patent application, filed by Iowa State University Research Foundation, Inc., Docket No: P11976US00.

## Krishna Jagadish SV, Kansas State University

**Objective 2 & 4:** Photosynthetic Capture and Photorespiratory Release of CO2 & Developmental and Environmental Limitations to Photosynthesis

**Impact Statement:** Minimize negative impact of heat and drought stress on the source-sink relationships of field crops

**Accomplishments:**

1. High night temperature induced post flowering change in carbon balance quantified (**wheat**)

2. Using GWAS, genomic regions responsible for light induced effective quantum yield for drought stress under field conditions identified (**rice**)

3. Temporal change in the chlorophyll content and effective quantum yield in flag leaves, spikes and awns documented (**winter wheat**)

4. Influence of changing diurnal temperature amplitude on photosynthesis and night respiration recorded (**corn**)

**Plans for Coming Year:**

* Identify genomic regions responsible for temporal change in light induced effective quantum yield, chlorophyll content under drought stress and control conditions (**sorghum**)
* Explore mechanistic responses of post flowering carbon balance exposed to high night temperature in contrasting **winter wheat** cultivars
* Validate the contribution of flag leaf, spike and awns photosynthetic contribution exposed to control and heat stress using field based heat tents (**winter wheat**)
* Lipid remodeling in **sorghum** leaves exposed to severe drought stress during vegetative stage

**Publications (2016):**

**John Sunoj, V.S., Shroyer, K.J., Jagadish, S.V.K., Vara Prasad, P.V.** (2016). Diurnal temperature amplitude alters physiological and growth response of maize (*Zea mays* L.) during vegetative stage. Environ. Exp. Bot. [**130**](http://www.sciencedirect.com/science/journal/00988472/130/supp/C), 113–121.

**Bahuguna, R., Solis, C., Shi, W., Jagadish, S.V.K.\*** (2016). Post-flowering night respiration and altered sink activity account for high night temperature-induced grain yield and quality loss in rice (*Oryza sativa* L.). Physiol. Plant. doi:10.1111/ppl.12485

**Wilkins, O., Hafemiester, C., Plessis, A., Holloway-Phillips, M.M., Pham, G., Nicotra, A.B., Gregorio, G.B., Jagadish, S.V.K., Septiningsih, E.M., Bonneau, R., Purugganan, M.** (2016). Environmental gene regulatory influence networks in rice (*Oryza sativa*) – response to water deficit, high temperature and agricultural environments. Plant Cell [http:/​/​dx.​doi.​org/​10.​1105/​tpc.​16.​00158](http://dx.doi.org/10.1105/tpc.16.00158)

**Jagadish, S.V.K, Bahuguna, R.N., Djanaguiraman, M., Gamuyao, R., Prasad, P.V.V., Craufurd, P.Q.** (2016). Implications of high temperature and elevated CO2 on flowering time in plants. Front. Plant Sci. doi: 10.3389/fpls.2016.00913.

**Vara Prasad, P.V., Bhemanahalli, R., Jagadish, S.V.K\*.** (2016). Field crops and the fear of heat stress - opportunities, challenges and future directions. Field Crops Res (In press)

**Shi, W., Xiao, G., Struik, P.C., Jagadish, K.S.V.\*, Yin, X\*.** (2016) Quantifying source-sink relationships of rice under high night-time temperature combined with two nitrogen levels. Field Crops Res. <http://dx.doi.org/10.1016/j.fcr.2016.05.013>

**Bheemanahalli, R., Sathishraj, R., Tack, J., Nalley, L.L., Muthurajan, R., Jagadish, K.S.V\*.** (2016). Temperature thresholds for spikelet sterility and associated warming impacts for sub-tropical rice. Agric. For. Meteorol. **221**, 122–130

*\*Corresponding author*

## JC Jang, Ohio State University

**Objective 3: Mechanisms Regulating Photosynthate Partitioning**

**Objective 4: Developmental and Environmental Limitations to Photosynthesis**

**Impact Statement:**

Tandem CCCH zinc finger (*TZF*) genes play pivotal roles in plant growth and stress response. Numerous reports have indicated that TZF proteins control seed germination, plant size, flowering time, and enhance biotic and abiotic stress responses via regulation of gene expression. Despite growing genetic evidence, the underlying molecular mechanisms are elusive. Jang lab has pioneered the functional analysis of plant *TZF* genes. They have recently demonstrated that plant TZF proteins can target specific RNA sequences and affect their stabilities. The current hypothesis is centered at the roles of plant TZF proteins mediated post-transcriptional regulation of gene expression.

**Accomplishments:**

1. Identification of AtTZF1 target RNAs using RNA immunoprecipitation coupled with RNA-seq.

2. Demonstrating that RR-TZFs interact and recruit stress responsive regulators to processing bodies and stress granules, presumably to facilitate mRNA degradation and translational repression.

3. Identification of upstream regulators of TZF-mediated processing-body and stress granule assembly.

4. Deciphering the roles of AtTZFs in plant abiotic defense response.

**Plans for Coming Year:**

* Genome-wide identification of Arabidopsis TZF1 mRNA targets
* In-depth characterization of Arabidopsis TZF1-RNA interaction
* Determine the mechanism underlying AtTZF1-mediated mRNA degradation
* P-body and stress granule dynamics under biotic and abiotic stresses

**Publications:**

Jang, J-C. (2016). Arginine-rich motif-tandem CCCH zinc finger proteins in plant stress responses and post-transcriptional regulation of gene expression. ***Plant Science*** 252:118-124. (Review).

Bogamuwa, S. and Jang, J-C. (2016). Plant tandem CCCH zinc finger proteins interact with ABA, drought, and stress response regulators in processing-bodies and stress granules. ***PLoS ONE*** 11(3): e0151574. Doi:10.1371/journal.pone.0151574.

Qu, J., Kang, S. G., Hah, C., and Jang, J-C. (2016). Molecular and cellular characterization of GA-stimulated transcripts GASA4 and GASA6 in *Arabidopsis thaliana*. ***Plant Science*** 246: 1-10.

## Helmut Kirchhoff, Institute of Biological Chemistry, Washington State University.

**Objective 1: Understanding architectural dynamics in plant thylakoid membranes**

**Impact Statement:** The architecture of photosynthetic thylakoid membranes in plants is highly dynamic, changing its shape in response to environmental cues. These structural dynamics of thylakoid membranes are the basis for the functionality, regulation, and maintenance of photosynthetic performance. Therefore, the knowledge of 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 in the resurrection plant, *Craterostigma pumilum*, (can withstand severe drought stress) the photosynthetic machinery under dehydration stress prevents damage induced by reactive oxygen production by a controlled shutdown of specific light reactions in the thylakoid membrane. A central role is the degradation of the cytochrome b6f complex.

2. We developed a new method for quantification of different photosystem II supercomplex assembly forms in thylakoid membranes (manuscript under review). This allows studying supercomplex dynamics in response to different environmental cues.

3. The stacking of thylakoid membranes to grana is a structural hallmark of photosynthetic membranes in plants. However, the factors that control membrane stacking are poorly understand. By calculating repulsive and attractive forces between membranes, we can predict how membranes stack. This study highlights the central role of membrane surface charges for stacking. Changes of membrane surface charges by reversible protein phosphorylation is a mechanism to modulate stacking and in-membrane protein-protein interactions. A manuscript is under review.

**Plans for Coming Year:**

* Determine factors that control dynamic swelling/shrinkage of the thylakoid lumen (focus on ion transporters) by using electron microscopy.
* Measuring the kinetics of architectural membrane changes induced by light.
* Determine how protein phosphorylation and the lipid/fatty acid composition control surpramolecular protein organization in grana.

**Publications (2015):**

**S. Tietz, S. Puthiyaveetil, H.M. Enlow, R. Yarbrough, M. Wood, D.A. Semchonok, T. Lowry, Z. Li, P. Jahns, E.J. Boekema, S. Lenhert, K.K. Niyogi, H. Kirchhoff** (2015) Functional Implications of Photosystem II Crystal Formation in Photosynthetic Membranes. *The Journal of Biological Chemistry* 290, 14091-14106

**D. Charuvi, R. Nevo, E. Shimoni, L. Naveh, A. Zia, Z. Adam, J.M. Farrant, H. Kirchhoff,** **Z. Reich** (2015) Photoprotection conferred by changes in photosynthetic protein levels and organization during dehydration of a homoiochlorophyllous resurrection plant. *Plant Physiology* 67, 1554-1565

**R. Hoehner**, H. Kirchhoff, **S. Puthiyaveetil** (2015) Structural and functional dynamics of the thylakoid membrane system. In: Chloroplasts: Current Research and Applications (Editor: H. Kirchhoff), Horizon Press, 59-88

## Karen E. Koch, University of Florida

**Objective 3: Mechanisms regulating photosynthate partitioning**

**Impact Statement:**

This year’s work on photosynthate import into grains addressed the dual goal of enhancing partitioning to yield as well as increasing movement of photosynthetic end-products out of leaves to prevent inhibitory build-up. Toward this end:

1) Our development of new molecular and genetic materials for public resources included over 9,000 new maize mutants, mapped insertion sites, and seeds for these UniformMu lines to the Maize Genetics Cooperative Stock Center. As of this year, these new lines, together with previous material, impacted almost 1,500 researchers worldwide, who requested and received over 22,000 UniformMu seed stocks.

2) Our sequencing of the W22 maize genome (as part of a seven-member consortium) is providing a much-needed molecular framework for the many researchers who use this inbred in their programs.

3) Analyses of selected maize mutants indicate new avenues for enhancing photosynthate partitioning to these grains may include genes for *Nucleotide Diphospho Kinase* (*Ndpk1*) and regulators of chromatin condensation (*Rcc1*).

**Accomplishments:**

1. We generated over 9,000 maize mutants for public use, mapped all insertion sites to the maize genome, and deposited stable research lines in the Maize Genetics Cooperative Stock Center. Approximately half the maize genome is now tagged with one or more mapped Mu transposons. Seeds for any mutant line can be identified (searchable by sequence) and requested at one MaizeGDB.org.

2. To more accurately map and use the mutants above (UniformMu population in the W22 background), we joined a seven-member consortium and sequenced the W22 maize genome. Assembly is completed. We re-mapped locales of all Mu insertions.

3. Protocols were developed for linking phenotype to genotype in maize using high-throughput methods with Mu-tagged materials (Peng et al., 2016).

4. Key mutants were selected and used to test hypotheses for roles of specific genes in photosynthate partitioning to developing maize kernels. A) One nucleoside diphospho kinase (NDPK1) emerged as potentially-central player in sucrose metabolism under the low-oxygen conditions that typify interiors of developing grains. B) A regulator of chromatin condensation (RCC1) was found critical for assimilate deposition of proteins in the developing grain.

5. Crystal structure of a maize alpha keto reductase (AKR) was compared to that of other family members to determine what allowed some of these enzymes to handle sugar substrates and not others (de Guiseppe et al., 2016)

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

**Publications (2015-2016):**

**de Giuseppe, P.O., dos Santos, M.L., de Sousa, S.M., Koch, K.E., Yunes, J.A., Aparicio, R., and Murakami, M.T.** (2016) A comparative structural analysis reveals distinctive features of co-factor binding and substrate specificity in plant aldo-keto reductases. Biochemical and biophysical research communications 474: 696-701.

**Liu, P., McCarty, D.R., and Koch, K.E.** (2016) Transposon mutagenesis and analysis of mutants in UniformMu maize (*Zea mays*). Curr. Protoc. Plant Biol.1:451-465. doi: 10.1002/cppb.20029

**Djidonou, D., Simonne, A.H., Koch, K.E., Brecht, J.K., and Zhao, X.** (2016) Nutritional quality of field-grown tomato fruit as affected by grafting with interspecific hybrid rootstocks. HortScience (accepted with revisions)

**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 47:1489–93.

**Other:**

* Executive Committee, Maize Genetics Cooperation (Elected for 2016-2020) (Vice Chair, 2017, Chair, 2018).
* National Secretary and Chair of the Program Committee for the American Society of Plant Biologists. (Elected for 2012-2016).
* Charles Reid Barnes Life Membership Award, American Society of Plant Biologists (2016-).
* Fellow of the American Society of Plant Biologists (2012-)
* Fellow of the American Association for the Advancement of Science (2012-)
* Google Scholar Citation Classic: >1,600 citations of Koch KE, Carbohydrate modulated gene expression. 1996. Ann Rev Plant Biol. 47: 509-540.

## Jiaxu Li, Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University

**Objective 4:** Developmental and Environmental Limitations to Photosynthesis

**Title:** Effect of silicon on the growth and drought response of soybean plants

**Impact Statement:**

Drought can strike soybean plants at any time during summer in southern states like Mississippi. Therefore, therefore is a need to develop production systems to maintain soybean yields on nonirrigated sites. Silicon application has been reported to increase water use efficiency and improve drought tolerance in several crops, but its beneficial effects are not known for soybeans. Our preliminary studies in the greenhouse indicate that application of potassium silicate to soil can improve drought tolerance in soybeans.

**Accomplishments:**

We evaluated the effects of silicon application on vegetative growth and drought tolerance of two soybean varieties grown in the greenhouse. During drought treatment, moisture index was higher in soil with than without potassium silicate supplement. Plants grown with potassium silicate had higher chlorophyll content than plants grown with potassium silicate under drought condition. Other parameters like leaf area, number of leaves, and number of roots were more in case of potassium silicate treated than the control plants. These results indicate that silicon application can improve the growth and drought tolerance of greenhouse-grown soybeans.

**Plans for Coming Year:**

We will evaluate the effects of silicon application on vegetative growth and seed yield of soybeans grown on nonirrigated sites.

**Publications (2016):**

**Sah, S.K., [Reddy, K.R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Reddy%20KR%5BAuthor%5D&cauthor=true&cauthor_uid=27200044)., and** [**Li, J**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27200044)**.** (2016). Abscisic acid and abiotic stress tolerance in crop plants. [Front Plant Sci.](http://www.ncbi.nlm.nih.gov/pubmed/27200044) **7**, 571.

## Anastasios Melis, Ph.D. University of California, Dept. of Plant & Microbial Biology, Berkeley, CA 94720-3102.

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**Objective # 3:** Mechanisms regulating photosynthate partitioning

**Impact Statement:**

The photosynthetic apparatus can be heterologously transformed for the generation of terpene hydrocarbons of commercial interest. However, the slow catalytic activity of target terpene synthases (kcat=4 s-1 or slower) and slow carbon flux through the methylerythritol-phosphate (MEP) pathway combine to limit rate and yield of product generation. As proof of concept in efforts to overcome these barriers, work in this paper applied transformation technologies in *Synechocystis* for the heterologous production of β-phellandrene (monoterpene) hydrocarbons. Conditions were defined whereby expression of the β-phellandrene synthase (PHLS), as a CpcB•PHLS fusion protein with the β-subunit of phycocyanin, accounted for up to 20% of total cellular protein. The high cellular concentration of the PHLS transgene compensated for the slow catalytic activity of this enzyme. Moreover, CpcB•PHLS was heterologously co-expressed with enzymes of the mevalonic acid (MVA) pathway and geranyl-diphosphate (GPP) synthase, increasing cellular carbon flux toward the terpenoid biosynthetic pathway and enhancing substrate availability to the PHLS. Such improvements enabled yields of 10 mg of βphellandrene per g of dry cell weight generated in the course of a 48 h culture incubation, the equivalent of 1% β-phellandrene:biomass (w:w) carbon partitioning ratio. **Accomplishments:**

The work helped identify prerequisites for the efficient heterologous production of terpene hydrocarbons by the photosynthetic apparatus: (i) requirement for overexpression of the heterologous terpene synthase, so as to compensate for the slow catalytic turnover of the enzyme, and (ii) enhanced endogenous carbon flux toward the terpenoid biosynthetic pathway, *e.g*. upon heterologous expression of the MVA pathway, thereby supplementing the native MEP C-flux toward the universal isopentenyldiphosphate and dimethylallyl-diphosphate terpenoid precursors. The two prerequisites are determinants of yield in the photosynthetic CO2 conversion to terpene hydrocarbons.

**Plans for Coming Year:**

We will continue to optimize plant terpene synthase and carbon flux gene expression with the objective of further improvements in the terpene-to-biomass (w:w) carbon partitioning ratio.

**Publications (2016):**

Formighieri C, Melis A (2016) Sustainable heterologous production of terpene hydrocarbons in cyanobacteria. Photosynth Res. DOI 10.1007/s11120-016-0233-2

## 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:**

1. 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 and non-competitively inhibit SSIII catalytic activity.

2. Artificial microRNA gene suppression of SBDCP restored normal RNA and protein expression levels of SSIIIa in over-producing ADPglucose transgenic rice lines resulting in starch with lower amylose content and increased amylopectin chains with higher degree of polymerization.

3. Artificial microRNA gene suppression of SBDCP also mediated a further 5% increase in grain weight but ADPglucose levels remained elevated suggesting additional barriers to starch synthesis.

4. The catalytic properties of starch phosphorylase Pho1 were enhanced at low temperature as KM for glucose-1P and amylopectin were significantly lower at 16°C than at 36°C.

5. Pho1 differs from non-plant phosphorylases in having an extra 78-80 amino acid peptide (L78 or L80) located at about the middle of the primary sequence. Removal of this L80 peptide in the rice enzyme had no significant effect on catalytic properties and regulation by ADPglucose.

6. Pho1 was found to interact with DpeI (disproportionating enzyme) to form a larger enzyme complex, which utilized a broader range of substrates for enhanced synthesis of larger malto-oligosaccharides than each individual enzyme and significantly elevated the substrate affinities of OsPho1 at 30°C. Moreover, the assembly with OsDpe1 enables OsPho1 to utilize products of transglucosylation reactions involving G1 to G3, sugars that it cannot catalyze directly.

7. Analysis of mutant lines for the plastid phosphoglucosmutase and ADPglucose pyrophosphorylase indicated that the primary location of ADPglucose synthesis in developing pollen was in the plastid. Moreover, starch deficient pollen were unable to successfully compete against starch containing pollen in fertilization.

7. The ER membrane protein Got1 was shown to be involved in the localization of prolamine and globulin mRNAs to the specific subdomains of the cortical-ER and trafficking of glutelin and globulin storage proteins to the protein storage vacuole.

**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 (2016):**

**Cakir, B., Shiraishi, S., Tuncel, A., Matsusaka, H., Satoh, R., Singh, S., Crofts, N., Hosaka, Y., Fujita, N., Hwang, S.-K., Satoh, H. and Okita, T.W.** (2016). Analysis of the Rice ADP-Glucose Transporter (OsBT1) Indicates the Presence of Regulatory Processes in the Amyloplast Stroma That Control ADP-Glucose Flux Into Starch. Plant Physiol. 170:1271-1283.

**Hwang, S.-K., Singh, S., Cakir, B., Satoh, H. and Okita, T.W.** (2016). The Plastidial Starch Phosphorylase From Rice Endosperm: Catalytic Properties at Low Temperature. Planta 243:999-1009.

**Hwang, S.-K., Koper, K., Satoh, H. and Okita, T.W.** (2016). Rice Endosperm Starch Phosphorylase (Pho1) Assembles with Disproportionating Enzyme (Dpe1) to Form a Protein Complex That Enhances Synthesis of Malto-Oligosaccharides. J. Biol. Chem. In press.

**Lee, S.-K.,Eom, J.-S., Hwang, S.-K., Shin, D.-J., An, G., Okita, T.W. and Jeon, J.-S.** (2016). Plastidic Phosphoglucomutase and ADP-Glucose Pyrophosphorylase Mutants Impair Starch Synthesis in Rice Pollens and Cause Male Sterility. J. Exp. Bot. In press.

**Fukuda, M., Kawagoe, Y., Murakami, T., Washida, H., Sugino, A., Nagamine, A., Okita, T.W., Ogawa, M. and Kumamaru, T.** (2016). The Dual Roles of the Golgi Transport 1 (GOT1B): RNA Localization to the Cortical Endoplasmic Reticulum and the Export of Proglutelin and α--Globulin from the Cortical-ER to the Golgi. Plant Cell Physiol. In press.

## Rebecca L. Roston, University of Nebraska-Lincoln

**Objective #1: Plastid Function and Intracellular Communication**

**Impact Statement:** This year we examined the relationship of the plastid with the rest of the cell during signaling of the plant-wide abiotic stress freezing. We discovered that the chloroplast, through SFR2 and unknown enzymes interprets freezing through changes in cytoplasmic pH and ion homeostasis, and alters chloroplast lipid levels. This work has been communicated through a publication and presentations at international meetings, and was performed in collaboration with Dr. Christoph Benning. Further, we have collaborated with the lab of Dr. Julie Stone to identify the sub-organellar location of DJ1C. We produced *Brassica rapa* DJ1C protein *in vitro*, and showed that it was only poorly imported to chloroplasts. Further experiments are required to determine why it fusions of DJ1C are located in the chloroplast. Finally, we have tested a number of constructs to generate fluorescent visualization mechanisms to better understand membrane transport from the inner envelope to the thylakoid membrane. The majority of these have been problematic and we are pursuing alternative options.

**Accomplishments:**

1. Determined at least two factors activating SFR2, a chloroplast membrane protein critical for freezing tolerance.

2. Determined that DJ1C is not imported into chloroplasts at detectable levels on a short timescale.

3. Generated multiple Arabidopsis lines with fluorescent reporters inside the chloroplast.

**Plans for Coming Year:**

* Expand our understanding of the role of SFR2 by screening phylogenetically diverse plants for the SFR2-specific lipid responses under freezing conditions.
* Pursue additional mechanisms by which SFR2 may be responding to freezing conditions.
* Refine efforts to visualize Arabidopsis chloroplast membrane transport using fluorescent reporters.

**Publications:**

**AC Barnes, Benning C, and Roston RL.** (2016) Chloroplast membrane remodeling during freezing stress is accompanied by cytoplasmic acidification activating SENSITIVE TO FREEZING 2. Plant Phys. **171,** 2140-2149.

**Warakanont J, Tsai C-H, Michel EJS, Murphy III G, Hsueh PY, Roston RL, Sears BB, and Benning C.** (2015) Chloroplast lipid transfer processes in *Chlamydomonas reinhardtii* involving a TRIGALACTOSYLDIACYLGLYCEROL 2 (TGD2) orthologue. Plant J. **84,** 1005-1020.

**Other:** Collaborations with Dr. Julie Stone’s and Dr. Christoph Benning’s lab.

## Thomas D. Sharkey and Sean E. Weise, Michigan State University AgBioResearch

**Objective 2: The Glucose-6-Phosphate Shunt Around the Calvin-Benson Cycle**

**Impact Statement: The glucose-6-phosphate (G6P) shunt may result in 10 to 20% carbon loss during normal photosynthesis. This disadvantage may be balanced by efficient resupply of intermediates to the Calvin-Benson cycle in a stochastic environment. Understanding the rate and regulation of the shunt may result in discovery of ways to improve carbon metabolism of photosynthesis.**

**Accomplishments:**

1. Characterized plastidial phosphoglucoisomerase, one of the sources of G6P for the shunt. PGI is extremely sensitive to competitive inhibition by erythrose 4 phosphate. The *K*m for G6P is higher than for fructose 6-phosphate suggesting that flow is primarily from F6P to G6P.

2. Engineered plants to have constitutive expression of glucose-6-phosphate transporter (GPT2), the other source of G6P for the shunt. These plants are very sensitive to increases in light level but grow reasonable when the growth light level is held constant.

3. Examined how the shunt could explain increased cyclic electron flow in a plant with reduced photorespiratory enzyme hydroxypyruvate reductase (HPR). 2-phosphoglycolate strongly inhibits triose phosphate isomerase. We found the triose phosphate were out of isomerase equilibrium in *hpr* plants. We propose that glyceraldehyde 3-phosphate is exported from the chloroplast and isomerized in the cytosol. Some of the carbon returns to the stroma through GPT2, whose mRNA is up in *hpr* plants. This leads to high G6P in the stroma, which stimulates the G6P shunt consuming ATP and stimulating cyclic electron flow to compensate the loss of ATP.

4. Examined gene expression for GPT2 and related transcription factors in response to a step change in light. Gene expression of several transcription factors increase 15 to 30 min after a switch to high light and GPT2 increases after one to two hours but falls back to low levels at four hours. In a tpt knock out plant GPT2 does not increase.

**Plans for Coming Year:**

* Measure the amount of G6P in the chloroplast under different conditions using non-aqueous fractionation
* Characterize mutants lacking GPT2 or overexpressing GPT2
* Make double mutants to make plants that make isoprene but lack starch synthesis to examine the speed of labeling of the Calvin-Benson cycle intermediates.

**Publications (2015-16):**

**Sharkey, T.D., and Weise, S.E.** (2016). The glucose 6-phosphate shunt around the Calvin-Benson Cycle. Journal of Experimental Botany **67,** 4067-4077.

**Other:**

none

## 2015 NC1200 Meeting Minutes:

**The NC1200 Annual Meeting was held at the Sheraton Westport Plaza in St. Louis, MO on November 7th, 2015.**

Meeting participants included:

Rob Aiken – Kansas State University

Christoph Benning – Michigan State University

Felix Fritschi – University of Missouri

Glenda Gillaspy – Virginia Tech

Michael Giroux – Montana State University

Jeffrey Harper – University of Nevada, Reno

Helmut Kirchhoff, - Washington State University

Tasios Melis – University of California, Berkley

Vara Prasad – Kansas State University

In addition to NC1200 members, the following prospective members also participated

Doug Allen - USDA-ARS, St. Louis

Krishna Jagadish - Kansas State University

**Business meeting:**

Presiding: Christoph Benning (Administrative Advisor for NC1200)

Topics:

1. Potential new members
2. Meeting location for 2016
3. NC1200 project information, renewal timeline and action items
4. Potential new Members

- Krishna Jagadish – participated in the meeting as a guest. Provided CV prior to the meeting that was circulated by e-mail.

Unanimously approved as a new member.

- Doug Allen – participated in the meeting as guest. Agreed to provide a CV to be circulated to the entire group. Vote by NC1200 members will occur through e-mail.

1. Meeting location for 2016

Locations including Washington DC, Charlotte, NC, and Bozeman, MT were discussed as potential sites. Bozeman was selected as the meeting site for 2016. Mike Giroux agreed to host the meeting. The date of the meeting is yet to be determined.

1. NC1200 project information, renewal timeline and action items:

Christoph Benning provided an overview and some background on the NCRA, North Central Regional Association of State Agricultural Experiment Station Directors. Information is available at [www.ncra.info](http://www.ncra.info), the Multi-state Research drop-down menu has a lot of relevant information.

The project summary of the previous project, NC1168 (2007-2012) is online at <http://www.nimss.org/projects/view/mrp/outline/8936>

The current project, NC1200, is approved for October 01, 2012 to September 30, 2017. (<http://www.nimss.org/projects/view/mrp/outline/14097>)

A discussion on the desire to prepare and submit a project renewal was discussed.

Meeting participants agreed to proceed with the preparation and submission of a renewal proposal.

The NCRA Timeline for project renewal is available at (<http://www.ncra.info/MSR_ApprovalProcess.php>)

Outline of the information at the above link:

NCRA Deadlines for NC1200 renewal

* September 15, 2016 – Deadline to submit a request to write a
* October 15, 2016 – Deadline to upload Objectives section in NIMSS
* November 15, 2016 – participants and AES offices have submitted Appendix E forms
* December 1, 2016 – Completed proposal due in NIMSS
* December 15, 2016 AA review forms due in NIMSS
* Late march/Early April, 2017 - Final project reviews and decisions made.
* June 1, 2017 – If necessary, proposal revisions completed
* Mid-July, 2017 – NCRA reviews revisions, if project is approved NC number will be assigned.
* September 30,2017 – old project expires
* October 1, 2017 - New project begins
* March 31, 2018 – Termination report for NC1200 due in NIMSS

Discussion ensued about the participants in NC1200 and project renewal. A number of NC1200 members have not participated in meetings in recent years. Some of these participants may choose not to be involved in a project renewal.

Christoph Benning agreed to continue to serve as Administrative Advisor for the renewal project. He called for volunteers to lead the writing process: Rob Aiken and Felix Fritschi agreed to serve on the writing committee and Christoph Benning agreed to serve in an advisory capacity. All members interested in participating are expected to contribute to the writing process.

The writing committee will send an e-mail to members of NC1200 to identify whether members wish to continue or discontinue participation in a renewal project.

Members interested to participate will be polled about existing and potential new objectives for the proposal. As part of this process, potential new members may be identified.

## Project Renewal Justification

**COOPERATIVE NORTH CENTRAL REGIONAL RESEARCH PROJECT**

**CURRENT PROJECT NUMBER: NC-1200**

**TITLE:** Regulation of Photosynthetic Processes

**DURATION:** **1 October 2017 - 30 September 2022**

**STATEMENT OF THE ISSUES AND JUSTIFICATION**

**Necessity of Photosynthesis Research**

Photosynthesis is essential to life on earth as it converts sunlight into biochemical energy used by all life forms. It is the primary process for generating plant biomass. As a result of photosynthesis, carbon dioxide, as well as inorganic nitrogen and sulfur, are converted into reduced forms of carbon, e.g. sugars, lipids, amino acids and other cell building blocks, and oxygen is produced through photosynthetic water oxidation. As such plant and algal photosynthesis affects global geochemical processes, in particular carbon cycling (42), and is an important factor to be considered in global climate change models. Aside from these fundamental aspects of photosynthesis, agricultural production of food, fiber~~s~~, natural chemicals, and biofuel feedstocks is directly affected by limitations in the rate and yield of photosynthesis (130,147). It is widely recognized that some of the greatest challenges humankind is currently facing --feeding an exponentially growing global population, supplying sufficient energy to sustain this global population, averting negative environmental impacts due to human activities-- can be addressed using photosynthetic organisms, i.e. agricultural crops, e.g. (39). Therefore, the need for state-of-the-art photosynthesis research to improve the efficiency of the process in traditional crops or for the development of novel crops has never been more urgent.

Collaborators participating in this regional project will place considerable focus on understanding and improving the response of photosynthesis to genetic, developmental and environmental factors that limit productivity. The research spans all levels of organization from the molecular and cellular through the leaf, whole plant and canopy levels. Particular emphasis will be placed on abiotic stresses (i.e., heat, cold, drought and salinity), nitrogen and water use efficiency, carbon flux pathways, and the signal transduction mechanisms that initiate the plant response. Factors that enhance or limit agricultural productivity generally do so by impacting photosynthesis. The gains in yield of the major crops over the past half-century have come about primarily from breeding for greater harvest index (HI) and through management, particularly the application of fertilizer (50). For well-fertilized crops, HI has approached the maximum achievable for many crops, and future gains will depend on increasing total production (127). Greater productivity, which is necessary to meet the food, fuel and fiber needs of a growing world population (103), requires improving the basic efficiency of the photosynthetic process for light energy capture and the utilization of this energy for the synthesis of organic molecules (127). For maximum benefit, the efficiency of the process must be increased under both optimal conditions and when conditions are sub-optimal, consonant with the changes in temperature~~s~~, CO2 and precipitation anticipated from climate change.

**Importance and Consequences**

It has become increasingly clear that global climatic trends are negatively impacting the yields of our major crops used for the production of food, feed and biofuels (83). Sustaining the productivity of traditional crops will require improvements in the efficiency of photosynthesis that go beyond traditional breeding and selection (94). Similarly, the development of novel feedstocks for biofuel/chemicals production in a way that is sustainable, that is not in competition with food, and that reduces greenhouse gas production requires novel approaches and non-traditional crops including possibly algae (118,134). Only a concerted and vertically integrated effort encompassing all aspects of photosynthesis by plant scientists will ensure that appropriate solutions will be found for some of these most pressing problems currently facing society. Innovative thinking will be required that does not stop at traditional agricultural systems and crops, but may enable transitioning to new crops dedicated to the production of biofuels and chemicals instead of food. As photosynthesis is at the basis of biomass production, we need to find innovative ways to overcome its limitations. Failure to do so now will limit our future ability to produce sufficient food and fuel~~s~~ in a rapidly changing climate.

**Technical Feasibility**

Genomic resources and second generation sequencing technologies have advanced to the point that a wealth of information can be generated for any species of plant or alga, e.g. date and oil palm (3,16), in a relative short time span and at reasonable costs. Thus, the raw material for genetic engineering of novel crops or algal strains is readily available. Large scale phenomics focused on chloroplast proteins in model plants such as Arabidopsis has become possible (85). Moreover, using comparative genomics, reconstruction of metabolism from gene expression and genomic data has become readily feasible for any organism. One example is the recent metabolic reconstruction of the alga *Chlamydomonas reinhardtii* (22). In addition, our knowledge and analysis of primary metabolism of plants and metabolic networks has advanced to levels (78) that can enable the rational design of novel crops, which will meet our needs. Despite this progress, the task of genetically transforming crop plants and analyzing the consequences (phenotypes) as the result of genetic engineering is still tedious and time consuming. Synthetic biology efforts with plants, involving stacking or replacing multiple genes, are lagging behind those for bacterial systems (98). Moreover, photosynthesis is one of the most complex processes found in nature, requiring hundreds of genes and proteins, and multiple and overlapping levels of regulation Thus, to enable rapid progress in the basic discovery process and to devise strategies for the improvement of photosynthesis in crops, facile model organisms such as Arabidopsis (121) or microalgae such as Chlamydomonas (99) will have to be employed to quickly identify the most promising directions for crop improvement. **Following this guidance, collaboration with geneticists and breeders, associating traits with genomic regions of mapping populations can support marker-assisted selection (Heffner et al., 2009).** Such an integrated approach requires multifaceted expertise and, thus, will benefit from synergy derived from a multi investigator effort.

**Multi State Effort**

Providing a conceptual framework through the current project, this North Central Regional Group of scientists has already successfully worked on diverse aspects of photosynthesis bringing together a complementary set of expertise. While global issues as laid out above are addressed, practical solutions to these problems often have local solutions, e.g. by taking into account climatic zones to which specific crop species or algae are adapted. Continued effort by the current group will contribute towards these main goals while also enabling local solutions of particular value to the North Central Region and other participating states. Participating partners are listed for each focus area below.

**Likely Impacts**

Efforts by the group are organized into four themes (Objectives). While the details and outcomes will be discussed below in the main body of the proposal, likely impacts falling under these themes can be briefly summarized as follows:

1. *Plastid Function and Intracellular Communication.* **Chloroplast plastids are the organelles that perform photosynthesis in both plants and algae.** It is also the location for a large number of enzymes turning this organelle into a biochemical production factory. **As an organelle, plastids do not function by themselves, but rely on extensive communication with other organelles within the cell, and with the whole plant.** The import of nuclear encoded proteins or membrane lipids assembled at the endoplasmic reticulum provide two examples (14,75). As primary photosynthate and many other metabolites, e.g. fatty acids, are only synthesized in plastids, they have to be exported to be available to other cell compartments. The integration of chloroplast biogenesis into overall cell development requires intricate signaling processes as does the adjustment of the photosynthetic electron transport chain to changing conditions. **Within the chloroplast photosynthesis occurs on a specialized structure called the thylakoid membrane, which is itself dynamically remodeled in response to development and stress cues. The architectural dynamics of the grana thylakoids are involved in regulating and maintaining photosynthetic performance. Studies will focus on how thylakoid membranes change their shape, the functional consequences of structural alterations, and effects of whole plant stresses and developmental cues on the thylakoid membrane and plastid lipid changes.** Likely impacts are a better understanding of **photosynthetic energy transformation, the development of the thylakoid membranes under developmental and stress regimens, and the development of tools that can be used to assess thylakoid and inner envelope connectivity** (NE-AES, VA-AES, WA-AES).
2. *Photosynthetic Capture and Photorespiratory Release of CO2.* Photosynthetic carbon fixation and photorespiratory release of CO2 have long been recognized as limitations to crop productivity (49,95,100,119). Considerable focus will be placed on engineering improvements in the organization of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the heat-sensitive **Rubisco** activase **(RCA)** that regulates its function. Microbial CO2 concentrating mechanisms will be investigated for potential improvements in the photosynthesis of algae and crop plants. **Studies will examine the mechanisms of RCA regulation in soybean including redox regulation, protein phosphorylation and possible ‘cross-talk’ between the two regulatory processes.** Emphasis will also be placed on the mechanisms that control carbon flux through primary and secondary metabolic pathways. Likely impacts will be insights into the regulation of carbon fixation and the generation of modified enzymes that improve primary and secondary carbon metabolism in plants. (CA-AES, IL-ARS, MI-AES, MO-AES, NV-AES, WA-AES).
3. *Mechanisms Regulating Photosynthate Partitioning.* Manipulation of carbon partitioning and understanding its regulation is central to **advances in yield formation in cereal and oilseed crops,** to the design of new crops for biofuel or commodity chemicals production that, e.g., divert carbon from carbohydrate synthesis to useful triacylglycerols (38), or terpenoid products (Reference). This work will lead to **the development and commercial application of novel organisms that are endowed with the heterologous expression of terpene synthases (from higher plants) in either production-type plants or microalgae.** The work will further seek to divert carbon-flux and to limit c**atabolic reactions resulting in respiration that reduces the fraction of photosynthate~~s~~ available for yield formation.** Transport phenomena at the cell, the tissue and whole plant level will be considered, sugar sensing mechanisms will be explored, as well as partitioning between storage carbohydrates, lipids and stress-protective compounds. Likely impacts will be defining the mechanisms that regulate photosynthate partitioning into the biosynthetic pathways for sucrose, starch, sugar alcohols, terpenoids, and lipids. Strategies will be developed to alter carbon partitioning by engineering bypass pathways **to overcome rate and yield barriers to commercial application**. (CA-AES, FL-AES, KS-AES, MI-AES, MO-ARS, MT-AES, NV-AES, WA-AES).
4. ***Developmental and*** *Environmental Limitations to Photosynthetic Productivity.* Factors such as leaf anatomy (126) or environmental stress-conditions ~~factors~~ such as high light (90), excess salinity or heat-stress (112) greatly affect photosynthesis**. Leaf stomata, regulating photosynthetic productivity as a ‘gateway’ for CO2 influx, are subject to complex active regulation. In the case of drought, the plant hormone abscisic acid (ABA) inhibits stomatal opening and promotes stomatal closure, supporting water conservation. A dynamic model of ABA-induced stomatal closure includes more than 40 identified network components (Li et al., 2006); additional plant water regulation is responsive to factors including light intensity, temperature, vapor pressure, and leaf CO2 partial pressure. Feedback regulation of photosynthesis by sugar sensing and signal transduction mechanisms are known, but incompletely understood. Frequently, these regulatory pathways result from a cascading sequence of processes.** Particular emphasis will be placed on identifying **processes driving responses to** abiotic stresses (temperature, water, and salinity), nitrogen use, and global atmospheric change. Likely impacts will be identifying **genomic regions associated with developmental and** environmental factors that influence photosynthetic productivity at the whole plant and canopy levels. (IL-AES, KS-AES, MO-AES, MS-AES, MT-AES, NV-AES, OH-AES).