THE WATER: NITROGEN TRADEOFF

Optimizing the Use of Water to Fix N and Reduce Agriculture's C Footprint

Grant Type: Multi-Investigator Project Team

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External Partners: Osito Orchard, Ela Family Farms, and the Colorado Fruit and Vegetable Growers Association

Modern agriculture is highly dependent on fertilizer made through fossil energy-intensive industrial N fixation. As energy prices and input costs increase, so does the price of N fertilizer. In recent years, high demand has led to fertilizer shortages, yet even more N fertilizer is needed to meet the demands of our crowded and hungry world.

Growing legumes in crop rotation is an option that farmers can use to benefit from biological N-fixation. However, very few farmers in the semi-arid West use cover crops due to their high water requirements. Biological N fixation by cyanobacteria provides an alternative means to supply N to crops while using less water, reducing input costs and increasing the fossil energy-efficiency of N fixation. Cyanobacteria use light energy to fix C and N from the atmosphere, and can, therefore, produce biologically-available N without other energy inputs such as natural gas. A distributed, on-farm N fixation system will provide additional benefits including reduced transport, decreased emissions, lower input costs, diversified income for farmers, and employment opportunities in rural communities. This type of system would initially lend itself to small and mid-size farms growing high-value crops such as fruits and vegetables, but could eventually spread to larger farms.

In contrast to approaches that have grown cyanobacteria for use as a soil inoculum, our research has focused on intensively growing cyanobacteria outdoors in high-rate ponds called raceways. We then irrigate directly with the cyanobacteria in liquid form, applying cyano-fertilizer directly through irrigation lines. There is some evaporative loss from production; in 2013 from 10-in (25 cm) deep raceways, we measured an average evaporative loss of 3.78 inches (9.6 cm) during each 2-week growing cycle. Total water loss depends on the surface area of the raceway, mixing characteristics, and climatic factors.

Cyanobacteria fix more N per unit of water than forage soybeans, red clover, and sweet clover, and about the same as hairy vetch (Table 1). In addition, much less land is needed for cyano-fertilizer production than for leguminous cover crops. Therefore, cyano-fertilizer is a viable, water-saving N fertilizer alternative to leguminous cover crops. Because of the ubiquity of cyanobacteria in nature, we chose to selectively enrich for local strains of N-fixing cyanobacteria. We hypothesized that these strains would be well adapted to local environmental conditions, and thus more competitive than non-indigenous strains. The cyanobacteria culture we have chosen to scale-up, *Anabaena* spp., has proven to be resistant to stresses and contamination in the lab and the field. Microorganisms that are grown under selective conditions that other organisms cannot tolerate are more resistant to contamination pressures. We have maintained a selective growing environment for our cyanobacteria because no C or N is supplied in the growth media. This has greatly reduced competitive pressure on our system since only N-fixing cyanobacteria are capable of fixing both C and N from the atmosphere.

Table 1. Water use efficiency and land use requirements of leguminous cover crops and cyano-fertilizer.

	Water Use Efficiency	Land Use Requirement
	--kg N/100,000 L water--	--ha N fixation/ha crops--
Forage soybeans	0.7	1.0
Red clover	1.1	1.0
Sweet clover	1.7	1.0
Cyano-fertilizer	3.4	0.09
Hairy vetch	3.7	1.0
Winter peas	6.1	1.0

The goal of this project is to develop a process through which farmers can make their own N fertilizer using cyanobacteria to fix N thus improving water use efficiency as compared to leguminous cover crops.

Objective 1: Refine operational parameters to enhance cyanobacterial production and N fixation per unit of water.

Based on our preliminary results regarding N use efficiency as compared to leguminous cover crops (Table 1), we aim to double the water use efficiency of cyanobacteria thus making it more efficient at fixing N than hairy vetch or winter peas. Healthy raceway cultures regularly produce 30 mg L^{-1} of total N during a 14-d batch production cycle. In 2014, we explored two methods for increasing production above baseline levels. The first incorporated delta wings to improve mixing and mass transfer in the raceways. The second introduced carbon dioxide (CO_2) into raceways as a supplemental source of carbon (C).

One of the key factors limiting scale up of microalgae cultures in open raceways is mixing (Grobbelaar, 2012). The primary benefit of increased mixing is the improvement of the mass transfer of nutrients, including CO_2, to the cyanobacteria, thereby increasing photosynthetic efficiency (Grobbelaar, 1994). The Utah State University (USU) raceway hydraulics group designed delta wings that improve vertical mixing (vs. the horizontal mixing provided by paddlewheels) (Voleti, 2012; Lance, 2012; Vaughan, 2013). The USU group determined that delta wings at a 40-degree angle, spanning the width of the raceway channel, and placed every 1.7 m could achieve sustained vortices throughout the raceway. When these delta wings were added to USU model raceways in a greenhouse, the biomass of *Chlorella vulgaris* (a green algae) increased by 27.1% compared with controls (Vaughan, 2013). We tested the USU delta wing configuration in an effort to achieve a 25% increase in *Anabaena* sp. biomass and total N.

N-fixing cyanobacteria require CO_2 for photosynthesis. Because of the difficulty in mass transfer between the production culture (liquid) and CO_2 in ambient air (gas), CO_2 in the production raceways will not be replenished naturally (Borowitzka, 2005). The fixation of dissolved inorganic carbon in the forms of CO_2 or H_2CO_3 also increases pH. *Anabaena* sp. continues to grow at high pH, and high pH can aid in the suppression of biological contaminants. However, it is likely that CO_2 is limited (Olaizola et al., 1991). Literature shows up to a four-fold increase in biomass with the introduction of low levels (.5-3%) of CO_2 (Olaizola et al., 1991; Arudchelvam and Nirmalakhandan, 2012). Our use of a N-fixing microorganism and a N-free growth medium likely reduces the upper limits of production that can be expected. Despite this, supplementation of CO_2 appears to have the best potential for increasing biomass and total N in our production cultures. We tested the supplementation of CO_2 in an *Anabaena* sp. N-fixing bacterial culture to determine if it could cost effectively increase biomass and total N during 14-d batch production.

METHODS

Field production experiments were conducted in the summer of 2014, at the CSU Horticultural Research Center in Fort Collins, CO. Six 9.9-m^2 open raceways with 25 cm depth were installed in 6.2m x 15.4m high tunnels. The RB growth medium, an organically certifiable medium (Barminski, 2014), was added to the raceway and allowed to mix prior to inoculation with *Anabaena* sp. cultures. To monitor the growth and health of the production culture, pH, temperature, and dissolved oxygen (DO) were measured daily between 2-4PM at three points within each raceway using an ORION 5 STAR portable meter (Thermo Scientific). Samples (15 mL) were collected at the same three data points within each raceway for measurement of optical density (OD). Every three to four days, additional samples were taken from the same sampling points for determination of Total Kjeldahl Nitrogen (TKN). A Hach DR 3900 Benchtop Spectrophotometer (Hach Company, Loveland, CO. USA) was used to measure OD (at 550 nm) and TKN. Statistical analysis was performed in R with $\alpha = .05$.

For the delta-wing trials, delta wings were designed using information from Vaughan (2013). A total of five two-delta arrays were placed 1.7 m apart along the channels of three raceways, except where the paddlewheel was attached. Three raceways served as controls. Two trials were conducted for mixing with delta wings. Due to high levels of algal contamination during the initial delta wings trial, conducted 6/25 to 7/9/2014, a second mixing trial was conducted 8/21 to 9/8/2014 using a healthier inoculum.

For the CO_2 treatment, three raceways were fitted with a PINPOINT pH Controller (American Marine, Inc.) to continuously monitor pH. A brass AC110V solenoid (Duda Energy, Alabama, U.S. Model 2W-200-20N) was attached to the pressure regulators on a 20-lb compressed CO_2 tank and plugged into the pH controller. CO_2 was bubbled into the raceway using 1.5 in x .5 in Sweetwater ceramic diffusers attached to ¼” ID x 3/8” OD ATP Vinyl-Flex clear plastic tubing. The pH controller for each raceway was set to open the solenoid when the pH reached 9.6 and bubble CO_2 until the pH reached 9.5.

RESULTS AND DISCUSSION

Delta Wings Mixing

In delta wings production trials, no significant differences were found at the end of production between raceways with delta wings and control for either OD or TKN (Table 2, Figure 1).

Table 2. Total Kjeldahl Nitrogen and Optical Density levels at the end of two batch experiments comparing raceways fitted with delta wings with control raceways. Data are means of 3 raceways (n=9).

	Deltas	Control	p-value (t test)
Trial 1 End of Batch (Day 14)			
Total Kjeldahl Nitrogen (mg L^{-1})	13.9	14.1	0.8928
Optical Density 550 nm (Abs)	0.293	0.314	0.3917
Trial 2 End of Batch (Day 18)			
Total Kjeldahl Nitrogen (mg L^{-1})	28.8	24.5	0.0807
Optical Density 550 nm (Abs)	0.333	0.312	0.0808

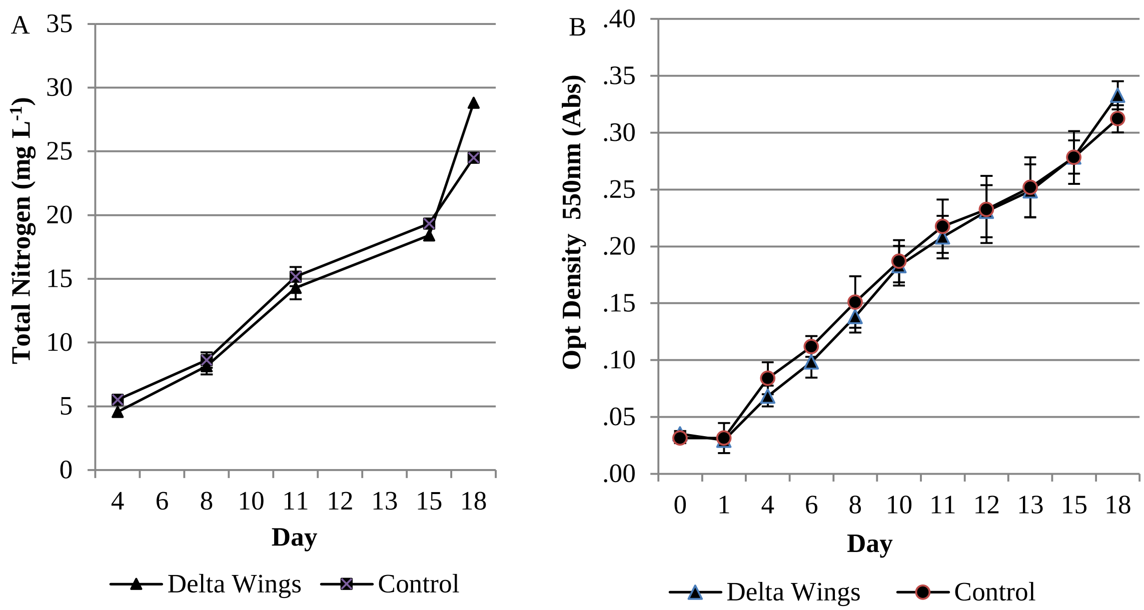

Figure 1. Total Kjeldahl Nitrogen (mg L^{-1}) (A) and Optical Density 550 nm (Abs) (B) as a function of time in a batch experiment comparing delta wings with control in 10 m^2 raceways (Trial 2).

CO_2 Supplementation

One control raceway (WSE) was excluded from statistical analysis as it was taken over by a *Scenedesmus* sp. alga shortly after inoculation. There was no significant difference in TKN between CO_2 supplemented and control raceways (Fig. 2). Raceways receiving CO_2 supplementation to maintain pH at a maximum of 9.5 began showing significant differences in OD vs. control on day 11 (Fig. 3). The pH was also significantly different in raceways receiving CO_2 most days after day 6, but not on the final day (day 18) of the production batch.

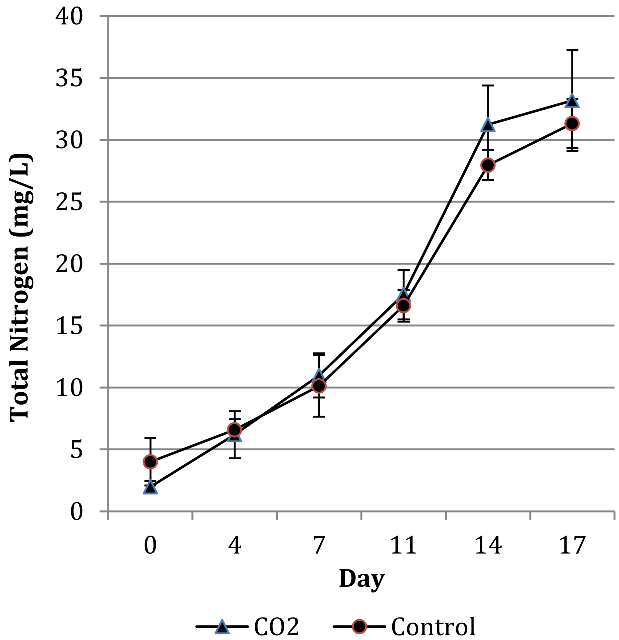

Figure 2. Total Kjeldahl Nitrogen (mg L^{-1}) as a function of time in a batch experiment comparing CO_2 supplementation to maintain a maximum pH of 9.5 with control in 10 m^2 raceways.

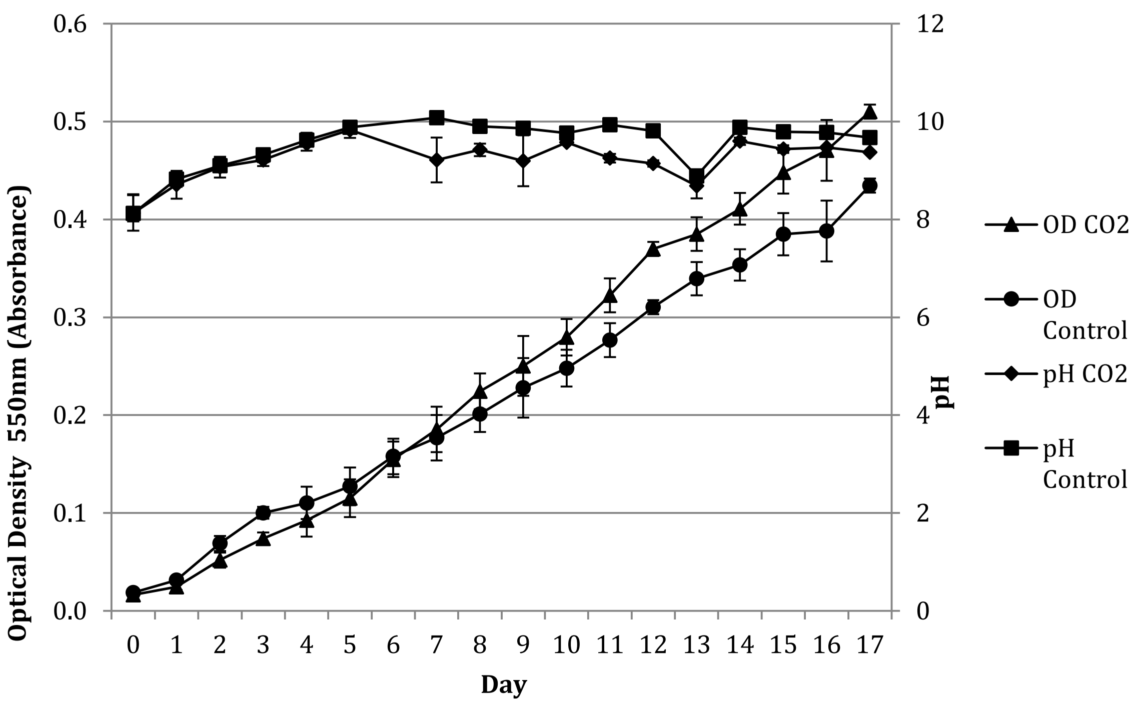

Figure 3. Optical Density 550 nm (Abs) and pH as a function of time in a batch experiment comparing CO_2 supplementation to maintain a maximum pH of 9.5 with control in 10 m^2 raceways.

Table 3. Effect of CO_2 Supplementation to Anabaena sp. Grown in 10 m^2 Raceways on Total Kjeldahl Nitrogen, Optical Density, and pH at the end of an 18-d Batch Experiment. Data are means of 3 raceways for treatment (n=9), and 2 for control (n=6)

	CO_2 to maintain max pH 9.5	Control	p-value (two sample t test)
Mean Total Kjeldahl Nitrogen (mg L^{-1})	31.2	31.3	.2473
Mean Optical Density 550nm (Abs)	.510	.435	<.001
Mean pH	9.37	9.67	<.001

In both delta wing trials, there was no significant difference in biomass or total N production between raceways with delta wings and the controls. It is possible that the spacing between delta wings within an array was too small, resulting in early deterioration of vortices. Another possibility is that the cultures have an overall nutrient limitation that cannot be addressed by improved mixing of the culture. Once the nutrient limitation (e.g., CO_2) is resolved, the delta wings could lead to enhanced growth, either by increasing overall biomass and total N or by increased growth rates and shorter production cycles.

During the CO_2 trial, beginning day 7 until the end of the batch, most days showed significant differences in pH between treatment and control raceways (Table 3). Overcast weather (notably day 13) reduced photosynthesis and pH in both control and CO_2 supplemented raceways (Fig. 3). Beginning on day 11, there were statistically significant differences in OD. However, there was no difference between total N in control raceways compared with pH controlled raceways. The increase in OD in supplemented raceways holds little practical significance, and it would be difficult to justify the costs of bubbling CO_2 to maintain a pH of 9.5.

Other nutrients may be limiting growth and N fixation. Increasing pH above 9.0 can render some nutrients, such as potassium and iron, unavailable to cyanobacteria (Evan and Prepas, 1997). Using Moreno et al. (2003) raceway production ranges of 9 g m^{-2} d^{-1} to 20 g m^{-2} d^{-1} as a reference for a marine N-fixing *Anabaena* sp., it could be reasonable to expect a 50% to 200% increase over our current baseline production. Moreno et al. (2003) maintained a pH between 8.5 and 9.0 to achieve those production levels, so it is possible that increasing CO_2 supplementation and reducing pH further to at least 9.0 could lead to cost effective increases in biomass and total N.

Objective 2: Communicate with farmers regarding the cyanobacterial bio-fertilizer production and utilization processes.

In addition to our research site at the Horticulture Research Farm, we are cooperating with two Colorado farms (Osito Orchard and Ela Family Farms) to test prototypes of the bio-fertilizer production system on-farm. We held field days at the CSU Horticulture Farm (August 27, 2014) and the private farms (Osito Orchard on July 15, 2014; and Ela Family Farms on July 24, 2014) during summer 2014. These field days served to not only tell farmers about our project but also to learn more about their production practices and challenges and get their feedback on our cyano-fertilizer approach.

To disseminate our research results to the scientific community, we made two scientific presentations at the annual American Society of Agronomy/Soil Science Society of America meeting (November 2014), one at the Organic Agriculture Research Symposium (February 2015), four at the Western Nutrient Management Conference (March 2015), and one at the American Society for Microbiology meeting (June 2015). In addition, we wrote two Extension articles disseminated through the CSU Extension system. We have initiated relationships with eOrganic and Appropriate Technology Transfer to Rural Areas to further disseminate our educational materials as they become available. Finally, we made five extension presentations (in addition to the field days described above) in CO, NM, OR, TX, and WI. These presentations and articles are listed in detail below in the Outcomes and Impacts section. They serve as first steps towards achieving the impacts planned for this project.

SUMMARY

Improving mechanical mixing and supplementing CO_2 remain the best candidates for economical increases in biomass and total N of nitrogen-fixing *Anabaena* sp. cultures. The differences in biomass late in the CO_2 supplementation experiment suggest that a pH ceiling of 9.5 may be the upper boundary for altering growth of our *Anabaena* sp. It could prove worthwhile to run the CO_2 supplementation experiments at levels that maintain incrementally lower pH values to 1) determine the efficacy of supplementation on cyanobacterial nitrogen fixation and 2) to find the most economical level of CO_2 supplementation for production of a N-fixing cyanobacteria-based fertilizer.

This study has shown that improved mixing may not be effective in a CO_2-limited culture. However, coupling the delta wings with CO_2 supplementation may yet result in improved nutrient transfer, photosynthetic efficiency, water use efficiency, and shorter fertilizer production times.

OUTCOMES AND IMPACTS

Proceedings

Sterle, D., G. Litus, F. Stonaker, S. Ela, and J.G. Davis. 2015. The effect of cyanobacteria biofertilizer on western Colorado organic peach quality and yield characteristics. Proc. of the Western Nutrient Management Conference; March 5-6 in Reno, NV.

Sukor, A., C. Ramsey, and J.G. Davis. 2015. Effects of commercial organic and cyanobacterial fertilizers on instantaneous water use efficiency in drip irrigated organic sweet corn. Proc. of the Western Nutrient Management Conference; March 5-6 in Reno, NV.

Wenz, J., H.N. Storteboom, and J.G. Davis. 2015. Effects of enhanced mixing and minimal CO_2 supplementation on biomass and nitrogen concentration in a nitrogen-fixing *Anabaena sp.* Cyanobacteria biofertilizer production culture. Proc. of the Western Nutrient Management Conference; March 5-6 in Reno, NV.

Wickham, A., and J.G. Davis. 2015. Effect of liquid organic fertilizers and seaweed extract on *Daucus carota var. Sativus* growth characteristics. Proc. of the Western Nutrient Management Conference; March 5-6 in Reno, NV.

Abstracts

Davis, J.G., H. Storteboom, and M.S. Massey. 2015. Developing an organic on-farm bio-fertilizer production system using cyanobacteria. Organic Agriculture Research Symposium; February 25-26, 2015 in Lacrosse, WI.

Sukor, A., and J.G. Davis. 2014. Influence of commercial organic and cyanobacterial fertilizers on yield and nitrogen use efficiency of lettuce and sweet corn. Paper 164-11. Soil Science Society of America Annual Meeting; Nov. 2-5, 2014 in Long Beach, CA.

Toonsiri, P. S.J. DelGrosso, J.G. Davis, A. Sukor, M. Smith, and M. Reyes-Fox. 2014. Comparison of organic fertilizer effects on nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions from a lettuce field. Paper 164-10. Soil Science Society of America Annual Meeting; Nov. 2-5, 2014 in Long Beach, CA.

Wenz, J., H. Storteboom, and J.G. Davis. 2015. Potential of a nitrogen-fixing *Anabaena* sp. cyanobacterium for use as a nitrogen biofertilizer. American Society for Microbiology; May 30-June 2, 2015 in New Orleans, LA.

Extension Articles

Davis, J.G. 2014. CSU Team Begins Testing of On-farm Bio-Fertilizer Production on Local Orchards. Fruit Facts May 2014. Colorado State University Western Colorado Research Center. Grand Junction, CO.

Davis, J.G. 2014. CSU Soil Fertility Team Testing On-Farm Bio-Fertilizer Production for High-Value Crops. Southeast Farm and Ranch Newsletter October 2014. Colorado State University Cooperative Extension Southeast Area. Lamar, CO.

Extension Presentations

Sterle, D., and J.G. Davis. 2015. On-farm Cyano-fertilizer Production and Use in West Slope Peach Orchards. Western Colorado Horticulture Conference, Grand Junction, CO, January 15, 2015.

Davis, J.G. 2015. Organic Fertilizer Comparisons, Texas Organic Farmers and Gardeners Association, San Antonio, TX, January 30-31, 2015.

Davis, J.G. 2015. On-farm Production and Use of Algae as Fertilizer, New Mexico Organic Farming Conference, Albuquerque, NM, February 20-21, 2015.

Davis, J.G. 2015. Developing an Organic On-Farm Bio-fertilizer Production System using Cyanobacteria, Organicology, Portland, OR, February 6-7, 2015.

Davis, J.G. 2015. Developing an Organic On-Farm Bio-fertilizer Production System using Cyanobacteria, Midwest Organic and Sustainable Education Service (MOSES) Organic Farming Conference, Lacrosse, WI, February 26-28, 2015.

Seminar

Davis, J.G., J. Wenz, H. Storteboom, A. Sukor, and P. Toonsiri. 2015. The Water Nitrogen Tradeoff: Optimizing the Use of Water to Fix N and Reduce Agriculture's Carbon Footprint. Colorado State University Water Seminar, Fort Collins, CO, March 9, 2015.

Leveraged Funding

Western Sustainable Agriculture Research & Education, On-Farm Cyanobacterial Bio-Fertilizer Production to Reduce the Carbon Footprint of Organic Fruit and Vegetable Production, $293,599, May 1, 2014--December 31, 2017.

Organic Farming Research Foundation, Effect of Organic Fertilizer Selection on Phytohormones, β-carotene Levels, Growth, and Yield of Carrots and Peppers, $14,000. January 1—December 31, 2015.

Submitted Proposal Still Under Review

USDA-Natural Resources Conservation Society-Conservation Innovation Grants, Harnessing the Sun to Produce Fertilizer On-farm to Achieve Energy Conservation, Air Quality, and Water Quantity Goals, $640,748, September 30, 2015—September 29, 2017.

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Voleti, R. 2012. Experimental Studies of Vertical Mixing in an Open Channel Raceway for Algae Biofuel Production. Master's thesis, Mechanical and Aerospace Engineering, Utah State University, Logan, UT.