Effect of Organic Fertilizer Selection on Phytohormones, β-carotene Levels, Growth, and Yield of Carrots and Peppers

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1. Project Summary

Supplying adequate nitrogen (N) to organic crops at the right time is a challenge. Our research team is developing a system for growing cyanobacterial bio-fertilizer (cyano-fertilizer) on farms and applying it through irrigation systems. Cyanobacteria are ubiquitous in soils and can fix N from the air using energy from the Sun through photosynthesis. Preliminary data from greenhouse studies has shown that cyano-fertilizer increased flowering and crop beta-carotene concentrations. The purpose of this project is to broaden this work to include fish emulsion (hydrolyzed and non-hydrolyzed) and kelp and evaluate their phytohormone concentrations and impact on nutritive value of organic crops. On-farm cyano-fertilizer production is an entirely new and innovative approach to providing crop N requirements using high-N bacterial biomass, while greatly reducing fertilizer manufacturing and transportation needs. In this project, on-farm cyano-fertilizer production and use were tested on a university research farm and two private orchards. The objectives of this project are listed below with a brief summary of our findings:

a) to quantify phytohormone concentrations (auxin and cytokinin, in particular) in organic fertilizers (including compost, fish emulsion, hydrolyzed fish emulsion, kelp, and cyanobacterial bio-fertilizer)

Six of the seven organic fertilizers analyzed were found to contain auxin and salicylic acid; however, none of the fertilizers contained any measurable levels of cytokinins. The non-hydrolyzed fish emulsion contained a high auxin content and a low salicylic acid content, while the hydrolyzed fish emulsion was relatively high in salicylic acid and low in auxin. The cyano-fertilizer was high in both phytohormones.

b) to assess the impact of phytohormones present in organic fertilizers on plant growth, yield, and quality of carrots and peppers (including β-carotene concentrations)

In both the field-grown carrot study and the greenhouse pepper experiment, the cyano-fertilizer and the non-hydrolyzed fish emulsion treatments had the highest crop yields. This may be due to both of these fertilizers resulting in the largest auxin application rates.

There was no fertilizer effect on beta-carotene concentration in peppers; however, the non-hydrolyzed fish emulsion had higher beta-carotene concentration compared to other organic fertilizer treatments when applied to lettuce. In the lettuce study, the auxin application rates were positively correlated with the beta-carotene concentrations.

In the pepper study, fertilizer treatment affected phytohormone levels in pepper leaves at harvest time. Specifically, the non-hydrolyzed fish treatment had higher abscisic acid and 12-oxo-phytodienoic acid concentrations in the leaves.

In addition, both foliar seaweed products resulted in a significant reduction in the number of bell-shaped peppers harvested.

c) to monitor the impact of cyanobacterial bio-fertilizer on peaches as compared to farmer's standard practice through on-farm, participatory research

In the on-farm research carried out on organic peach orchards, application of cyano-fertilizer in addition to compost increased peach yield and reduced the growth of the tree trunks. In addition, cyano-fertilizer increased the SPAD chlorophyll readings of the leaves, and the SPAD readings were positively correlated to the distal leaf iron concentration.

d) to quantify the direct costs and benefits of on-farm production and utilization of bio-fertilizer to optimize economic returns for organic farmers

The economic evaluation of the on-farm cyano-fertilizer production system found that cyano-fertilizer is already competitive with the most expensive organic N fertilizers. However, to be competitive with fish emulsions and blood and feather meals, the cost per lb of N must be reduced to about half of current costs. Based on smaller-scale experiments with supplemental CO_2, it is likely that we will be able to double N fixation while increasing costs by only about 20%. We will be evaluating this approach in field-scale raceways during the summer of 2016.

e) to impact farmer decision-making regarding fertilizer selection by sharing results through multiple methods

Eighteen presentations were made to farmer audiences and other conferences to disseminate the results of these studies.

2) Introduction to Topic

In order to target our research to stakeholder needs, attendees at the Colorado Big and Small Conference and New Mexico Organic Conference were surveyed regarding their soil fertility practices. Fifty-three farmers were surveyed representing CO (20), NM (28), and AZ (5). Farm size ranged from less than 1 acre to 1900 acres. Thirteen farms were certified organic, 26 considered themselves organic but not certified, 10 were conventional, 1 was biodynamic, and 3 gave no answer. Respondents use a variety of N fertilizers: 43% compost, 40% manure, 26% cover crops/green manures, 19% fish emulsion, 11% kelp, 9% blood meal, and 4% worm castings. On average, farmers spent $110/acre on fertilizers; however, the range was quite broad, from zero to $1,000/acre. Prices per pound of N (total N) are variable, with kelp over $1,000/lb N, and seabird guano and blood and feather meals at <$10/lb N. We used this information to guide our research into fertilizer effects that go beyond the nutrient concentrations in fertilizer to include potential impacts of phytohormones and to consider potential fertilizer impacts on crop nutrient concentrations in fertilizer decision-making.

Surveyed farmers also described the problems they face in meeting the nutrient needs of their crops with soil amendments and fertilizers. Responses included: inadequate nutrients, expense, inconsistent quality, application cost (equipment and labor), salinization, inadequate organic matter content, bulkiness of low nutrient materials (e.g., manures), dispersal of weed seed in manure, odor, sustainability (e.g., fish emulsion), and inadequate composting space. On a scale of 1-10, farmers were moderately satisfied with their soil fertility practices (6.8) but were also interested in new approaches (7.8). Surveyed farmers (91%) strongly preferred producing their own fertilizers over purchasing them. This knowledge guided us as we developed an on-farm production system to grow-your-own cyanobacterial bio-fertilizer and now as we test the impact of this fertilizer on a variety of horticultural crops.

Organic nitrogen fertilizers face serious limitations to their sustainability. Manure and compost are commonly used in organic farming systems, but they often come from off-farm, are low in N content, have to be hauled long distances, and can contribute to soil salinization. Since all manure is considered organic, antibiotics and hormones used in conventional animal agriculture can be transferred to organic farms through applied manure. Legume cover crops consume soil water while improving soil fertility, and are not commonly used in semi-arid areas due to the scarcity of water that could otherwise be used for cash crops. Many organic farmers use fertilizers that are hauled long distances (e.g., fish emulsion, guano) and are expensive (compared to manure). The processing and transportation involved increases the carbon footprint of organic crops. Most consumers of local, organic crops don’t realize that these local farms are often dependent on fertilizers shipped to them over considerable distances.

Cyanobacterial bio-fertilizer addresses many of these limitations to sustainability. We have been developing a biological fertilizer production process that can be carried out on farm, thus eliminating transportation requirements. In contrast to heterotrophic N-fixing bacteria (e.g. Azotobacter and Azospirillum), cyanobacteria are phototrophic N-fixers. By using the energy from the Sun as their sole energy source, cyanobacteria fix N while reducing the use of fossil fuels in fertilizer production. The nitrogen in cyanobacterial bio-fertilizer can help farmers fill seasonal N needs in the same way that fish emulsion is currently used, but using a fertilizer produced on-farm.

3) Objectives Statement

a) to quantify phytohormone concentrations (auxin and cytokinin, in particular) in organic fertilizers

Measureable Outcome: a table of auxin and cytokinin levels and the auxin: cytokinin ratio in at least six different organic fertilizers.

b) to assess the impact of phytohormones present in organic fertilizers on plant growth, yield, and quality of carrots and peppers (including β-carotene concentrations)

Measureable Outcome: an evaluation of whether fertilizers affected plant growth and yield differently due to differences in phytohormone concentrations in the fertilizers

The peppers were hailed out, and since it was getting too late to pant peppers outside, we decided to grow them in a greenhouse study which is reported below under this objective.

c) to monitor the impact of cyanobacterial bio-fertilizer on peaches as compared to farmer's standard practice through on-farm, participatory research

Measurable Outcome: an assessment of the effect of cyanobacterial bio-fertilizer on peach tree growth and yield

d) to quantify the direct costs and benefits of on-farm production and utilization of bio-fertilizer to optimize economic returns for organic farmers

Measureable Outcome: an evaluation of economic feasibility of on-farm production and use of cyanobacterial bio-fertilizer

e) to impact farmer decision-making regarding fertilizer selection by sharing results through multiple methods

Measureable Outcome: enhanced farmer knowledge regarding the presence of phytohormones in organic fertilizers and their effects on crop growth and beta-carotene levels

4) Materials and Methods

a. to quantify phytohormone concentrations (auxin and cytokinin, in particular) in organic fertilizers

We sampled a variety of organic fertilizers including fish emulsion, hydrolyzed fish emulsion, two seaweed-based solutions, blood meal, feather meal, and cyanobacterial bio-fertilizer and analyzed them for auxins, cytokinins, and other phytohormones. Auxin plays an important role in stem and root elongation and development of plant organs (such as leaves or flowers). Cytokinins promote cell division in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect bud growth and leaf senescence.

Phytohormone analyses were conducted at the Proteomics and Metabolomics Facility at Colorado State University. Fertilizer samples were adjusted to pH 7.0 with 1 N NaOH and extracted three times with water-saturated n-butanol followed by vacuum drying (Pan et al., 2010). The extracts obtained were filtered through membrane filters (pore size 0.45 µm). Supernatants were harvested by centrifugation at 5,000 g for 20 minutes at 4^oC. Supernatants were homogenized in liquid nitrogen using a cold mortar and pestle at 4^oC. The resulting supernatant was extracted using 80% methanol containing 10 mg L^{-1} butylated hydroxytoluene at 4^oC. Samples were methylated with diazomethane and dissolved in heptane. Gas chromatography-mass spectrometry (GC-MS) analyses were performed according to Edlund et al. (1995).

b. *to assess the impact of phytohormones present in organic fertilizers on plant growth, yield, and quality of carrots and peppers (including β-carotene concentrations)*

Certified organic land on the CSU Horticulture Field Research Center was utilized in this study. The CSU Horticulture Field Research Center has 10 acres of certified organic land. The Colorado Department of Agriculture is the certifier, and the land has been certified organic since 2002.

Effects of common organic fertilizers as well as organic cyano-fertilizer on carrot (*Daucus carota var. sativus*) growth and yield characteristics were tested during field experiments at the Horticulture Field Research Center in Fort Collins, CO in 2014 and 2015. Hydrolyzed and non-hydrolyzed fish fertilizer and cyano-fertilizer treatments were applied at prescribed nitrogen rates and distributed throughout the growth period approximately every 10 days. Control treatments received no supplemental N. Each treatment, including the control, was repeated with the addition of concentrated organic seaweed extract applied foliarly; Seacom PGR Seaweed was utilized in 2014, but we switched to Neptune's Harvest Seaweed in 2015 since we could not verify the organic certification of the Seacom product. Seaweed extract was applied at the manufacturers' recommended rates.

Bell peppers (*Capsicum annuum*) were grown in a greenhouse experiment in 2015 at the Colorado State University Plant Growth Facility. Hydrolyzed and non-hydrolyzed fish fertilizer, and cyano-fertilizer treatments were applied at 27 lb N $acre^{-1}$ in split applications approximately every 7 days over a 135 day growing period. Control plants received no supplemental N. Each treatment, including the control, was repeated with the addition of one of two liquid organic seaweed extracts applied foliarly. Foliar seaweed treatments were applied at the manufacturers' recommended dilution rates, but both brands were applied to evenly coat all pepper leaves.

A two-year field study was conducted on lettuce in the summers of 2013 and 2014 on certified organic land at the Colorado State University Horticulture Research Center, Fort Collins, CO. The aim of this study was to evaluate the effect of different types of organic N fertilizer on β-carotene concentration of lettuce. The fertilizers used in this study were blood meal, feather meal, non-hydrolyzed fish emulsion, and cyano-fertilizer. All fertilizers were applied at 50 lb N/acre. Both fish emulsion and cyano-fertilizer were supplied in four split applications over the growing season through drip irrigation, while the blood meal and feather meal were subsurface banded prior to planting.

c. *to monitor the impact of cyanobacterial bio-fertilizer on peaches as compared to farmer's standard practice through on-farm, participatory research*

Mature peach orchards were selected for use at Osito Orchard (Farm A) and Ela Family Farms (Farm B). Ela Family Farms has been certified organic since 2003, and Osito Orchard is transitional and will be certified in 2016. Both are certified by the Colorado Department of Agriculture. On both farms, the peach cultivar used was Sun Crest grown on a Lovell rootstock. The trees at Farms A and B were planted in 2008 and 1999, respectively.

Experimental plots consisted of five adjacent trees in the same row, with the entire plot receiving the treatment, but measurements were only taken from the three central trees. At farm A, two treatments were applied. The first treatment, "High Manure," was 100 lb N/acre as the dried chicken manure fertilizer True Organic 12-3-0 (Spreckels, CA) which was the grower's typical N fertilizer, made from a combination of feather meal, meat, and bone meal. A second treatment, "High Manure+Cyano," was 100 lb N/acre True Organic 12-3-0 with 10 lb N/acre from cyano-fertilizer. During summer of 2014, the trees at Farm A were heavily infested with green peach aphid (*Myzus persicae*), which damaged or killed many first year branches and severely lowered fruit counts and yields in some trees. Then, on April 3, 2015, a freezing event killed most of the blossoms on the trees, and it was decided to discontinue the experiment at Farm A.

At farm B, three treatments were applied: 1) "High Manure" was 100 lb N/acre from Richlawn 5-3-2 (Platteville, CO) a dried poultry manure, 2) "High Manure+Cyano" was 100 lb N/acre from Richlawn 5-3-2 with 4.5 lb N/acre from cyano-fertilizer, and 3) "Low Manure+Cyano" was 75 lb N/acre from Richlawn 5-3-2 with 4.5 lb N/acre from cyano-fertilizer. At farm B in 2015, a second group of treatments was added, comprised of the same treatment applications, but on new plots, within the same orchard. In 2015 the plots from the Farm B 2014 treatments continued to be evaluated for residual effects, but they were only given their respective amounts of Richlawn 5-3-2, excluding the cyano-fertilizer. On April 3, 2015 a freezing event killed most of the blossoms on the trees in the orchard, resulting in roughly 65% yield and fruit count reductions, since temperatures dropped to approximately -3.8 degrees C for a period of at least 3 hours.

Tree trunk cross sectional area (TCSA) was measured in the spring prior to fertilizer applications, and in the autumn after the growing season had ended. Circumferences were measured at a height of 21.3 cm from the orchard floor. Circumferences were then converted into TCSA by the formula: TCSA= $(\text{Trunk circumference}/2\pi)^2 \times \pi$. TCSA change was calculated by the formula: TCSA change= (End of season TCSA- beginning of season TCSA).

Chlorosis was monitored because moderate to severe chlorosis was evident in 2015. To quantify chlorosis, leaf chlorophyll was estimated using a SPAD502-PLUS meter (Konica Minolta, Osaka Japan) on first year, fully expanded leaves from the middle to the growing tip on all trees at Farm B on August 29, 2015. Each tree was divided into 4 quadrants based on compass directions. The mean of 5 measurements was recorded for each quadrant of each tree.

On Farm B in 2014, peaches were harvested by picking crews, in two rounds on August 20, 2014 and August 27, 2014, with all fruit being harvested by the end of the second picking. In 2015 on

Farm B, all peaches were harvested August 17, 2015. Peaches were harvested into boxes, and total fruit count and fruit weight were measured for each plot.

d. to quantify the direct costs and benefits of on-farm production and utilization of bio-fertilizer to optimize economic returns for organic farmers

Enterprise budgets were used to estimate material and labor costs of cyanobacterial bio-fertilizer production. The impact of variable labor costs on construction of cyanobacterial production raceways was evaluated using different scenarios. Total cost of cyanobacterial bio-fertilizer production was compared to that of commonly-used organic fertilizers. Cost estimates were run over a 5-yr period in order to factor in replacement costs for worn parts. Economic benefits were quantified by considering the reduction in off-farm fertilizer inputs and the impacts on crop yield and quality. Cost-benefit ratios were calculated to compare on-farm production of cyanobacterial bio-fertilizer with off-farm, organic sources. Finally, time to breakeven was calculated to estimate economic feasibility of on-farm cyanobacterial bio-fertilizer production.

e. to impact farmer decision-making regarding fertilizer selection by sharing results through multiple methods

Activities are described under Outreach below.

5. Project Results

Objective a. To quantify phytohormone concentrations (auxin and cytokinin, in particular) in organic fertilizers

Cytokinins were not detected in any of the seven fertilizers tested (Table 1). On the other hand, auxin and salicylic acid were detected in almost all of the fertilizers evaluated. However, no phytohormones were detected in Neptune's Harvest Seaweed.

Since fertilizers were applied at different rates in order to meet the same N application need, we also calculated the hormone application rates based on a common N application rate (62 lb N/acre). The non-hydrolyzed fish fertilizer had the highest auxin application rate but the lowest salicylic acid application rate (of the fertilizers that had detectable levels). Cyano-fertilizer had the highest salicylic acid application rate and the second highest auxin application rate. Hydrolyzed fish had the second highest salicylic acid application rate, but was among the lowest in auxin application rate. Seacom seaweed had the lowest auxin application rate and was moderate in salicylic acid application. The feather meal and blood meal fertilizers also contained both auxin and salicylic acid.

Table 1. Detected phytohormone concentrations in fertilizers used in field and greenhouse studies and phytohormone application rates (based on a fertilizer application rate of 62 lb N/acre).

Fertilizer	Phytohormone Concentrations			Phytohormone Application Rates		
	Auxin	Salicylic Acid	Cytokinin	Auxin	Salicylic Acid	Cytokinin
	------------------mg kg^{-1}------------------			------------------mg ha^{-1}------------------		
Cyano-fertilizer	6.50 x E-05	5.92 x E-03	n/d	207	20,000	n/d
Hydrolyzed Fish (Neptune's 2-4-1)	3.97 x E-04	0.018	n/d	1.34	259	n/d
Non-hydrolyzed Fish (Alaska 5-1-1)	1.436	0.077	n/d	1970	24.7	n/d
SeaCom PGR Seaweed (0-4-4)*	0.802	48.17	n/d**	0.962	57.8	n/d**
Neptune's Harvest Seaweed (0-0-1)*	n/d	n/d	n/d	n/d	n/d	n/d
Feather Meal (Down to Earth 12-0-0)	0.241	0.240	n/d	136	136	n/d
Blood Meal (Down to Earth (12-0-0)	0.058	0.050	n/d	32.9	28.4	n/d

*Seaweed N concentration=0, so seaweed treatments were applied following the manufacturers' recommendations.
**No cytokinin isomers were detected in the phytohormone assay, but the manufacturer reported 400 mg/kg cytokinins.

Objective b. To assess the impact of phytohormones present in organic fertilizers on plant growth, yield, and quality of carrots and peppers (including β-carotene concentrations)

Carrots

When comparing N fertilizer treatments in the carrot field study, no significant differences were detected in plant height, fresh and dry weight of plant parts, leaf to stem ratios, or total biomass. In 2014, cyano-fertilizer and non-hydrolyzed fish fertilizer yielded more than the control group (Figure 1). Hydrolyzed fish fertilizer performed similarly to the control group and other N fertilizers. Cyano-fertilizer also produced longer carrots than the control group in 2014 (but not in 2015). In 2015, cyano-fertilizer produced a greater yield than hydrolyzed fish fertilizer, but all treatments were comparable to the control. Cyano-fertilizer performed as well as, or better than the control group and other fertilizers in nearly all aspects measured, suggesting that cyano-fertilizer could be used in place of commonly used organic N fertilizers. Additionally, the foliar seaweed treatment had no effect on plant growth or yield in either year.

Peppers

All treatments produced pepper plants with similar leaf to stem ratios by weight, leaf and stem fresh weights, leaf and stem dry weights, and leaf numbers. Plants treated with cyano-fertilizer and non-hydrolyzed fish fertilizer produced 12.2% and 13.3% more yield than the unfertilized control (Figure 2). The N fertilizers also impacted the total number of branches, as the control plant had significantly fewer branches than all other N treatments. Foliar seaweed applications did not affect yield but they did result in pepper plants with differing branching height (distance to first branch) to total plant height ratios. Foliar seaweed treatments also presented significant differences in the total number of branches on each plant.

Foliar seaweed applications impacted pepper color and shape. The PGR Foliar Seaweed application significantly increased the number of green fruits while having no effect on the number of red fruits. Neptune's Harvest Foliar Seaweed was moderate (but not significantly different) in number of green peppers harvested as compared to the Control and the PGR Foliar Seaweed. In addition, the pepper shapes were described as bell-shaped, curved, or long, and both foliar seaweed products increased the number of long peppers while reducing the number of bell-shaped peppers. Finally, the PGR Foliar Seaweed also increased the number of curved peppers. Neither long nor curved peppers are the preferred type in the marketplace, and, therefore, care should be taken in applying seaweed products to bell peppers.

The leaves from plants treated with non-hydrolyzed fish fertilizer contained the highest concentrations of abscisic acid (phytohormone that impacts stomatal opening and closing) and 12-oxo-phytodienoic acid (a phytohormone that affects plant response to wounding)(Table 2). There were no significant differences in fruit β–carotene levels among treatments.

Table 2. Phytohormone concentration of bell pepper leaves from plants grown in a greenhouse experiment in Fort Collins, CO in 2015.

	Nitrogen Fertilizer			
Phytohormone	Control	Cyano-fertilizer	Hydrolyzed Fish Fertilizer	Non-Hydrolyzed Fish Fertilizer
abscisic acid (pg/mg)	21.4778 B	41.0444 AB	76.2667 AB	131.76 A
12-oxo-phytodienoic acid	294.56 AB	189.22 B	299.89 AB	350.00 A

Lettuce

In the lettuce field study, all fertilizer treatments in 2013 increased β-carotene concentration in leaf tissue compared to control, while only non-hydrolyzed fish emulsion recorded significantly higher β-carotene concentration compared to other treatments in 2014 (Figure 3). The high auxin application in the non-hydrolyzed fish emulsion treatment (see Table 1) could have increased β-carotene concentration in lettuce in both years. Auxin application rate was positively correlated with β-carotene concentration in 2013 ($r = 0.49$; $P = 0.0083$) and 2014 ($r = 0.53$, $P = 0.0029$). In addition, a significant negative correlation ($r = -0.7565$, $P=0.0044$) was found between marketable yield and β-carotene concentration in leaf tissue in 2014.

Objective c. To monitor the impact of cyanobacterial bio-fertilizer on peaches as compared to farmer's standard practice through on-farm, participatory research

The results at Farm B in 2014 showed increases in yield (Figure 4) and decreases in TCSA growth (Figure 5) in Cyano-Manure treatments compared to the No-Cyano manure treatments. It is likely that the lower TCSA growth followed the higher fruit count, because of the nutrient allocation being directed to the fruit (and away from the trunk) to a greater degree. The No-Cyano treatment had lower yields, but greater TCSA growth, indicating that lower fruit set may contribute to greater vegetative growth. In 2015 there were no significant differences found in fruit yield, average fruit count, or TCSA among treatments, possibly due to the impact of the freezing event during bloom.

However, in 2015, SPAD chlorophyll readings were significantly higher ($P=0.0350$) in Cyano-Manure treatments compared to the No-Cyano treatment. SPAD was significantly and positively correlated to distal leaf Fe concentration ($R=0.571$, $P=0.0136$). The effect of leaf Fe concentrations on chlorophyll content is well established (Abadia and Abadia, 1993; Belkhodja et al., 1998). Siderophores are sometimes released by cyanobacteria in response to Fe limiting environments (Wilhelm and Trick, 1994), and may have been applied with the cyano-fertilizer, causing increased Fe uptake.

Objective d. To quantify the direct costs and benefits of on-farm production and utilization of bio-fertilizer to optimize economic returns for organic farmers

The cost of materials dominated the total cost of raceway construction; materials were ~83% of the total cost ($1163 for a 6 ft x 36 ft raceway), while less than 20% of the cost was for labor. The 1st-year fertilizer cost savings in our experiments was not adequate to cover the raceway construction costs. Therefore, we are now aiming to reduce construction costs and increase raceway productivity in order to increase the value proposition for on-farm production of cyanobacterial bio-fertilizer. Based on work by other experts in micro-algae production, as we scale up we should be able to achieve raceway construction costs of \$5-10/m^2, costs much lower than our current raceways (\$26/m^2).

Operating costs for a 7-month production period (fifteen 2-wk cycles) are estimated to be $353 ($196 for electricity and $157 for nutrient solution). This does not include the opportunity cost of about 3 hours/week (90 hrs over the entire production period) of farmer management of the bio-fertilizer production process. Our calculations also do not include the labor and fuel savings achieved by applying cyanobacterial bio-fertilizer (or fish emulsion) through an irrigation system, as opposed to soil application and incorporation.

We developed scenarios to evaluate the impact of variable labor costs on total raceway construction costs. Although the amount of labor needed for raceway construction is fixed, the rate of pay is variable. Changing labor costs from $10/hr up to $30/hr, although a 3-fold increase, only results in ± 8.7% in construction cost. Therefore, the minimization of material costs (fixed costs) is most important in achieving shorter breakeven periods.

Alternative fertilizer costs are important to the profitability of on-farm cyanobacterial bio-fertilizer production. The higher the costs of the alternative fertilizers, the easier it is to achieve profitability of the cyanobacterial bio-fertilizer. Variability in the price of different organic fertilizers is high; organic fertilizers vary almost 5-fold from the cheapest to the most expensive (Table 3). Therefore, although profitability can already be shown in comparison to the most expensive organic fertilizers (e.g., alfalfa meal), we are focusing on reducing construction costs and increasing cyanobacterial growth and N fixation in order to be able to compete with the cheaper organic fertilizers. The shipping costs range from 33.3-60.0% of the total costs of organic fertilizers, respectively. In general, the larger the amount purchased, the lower the shipping cost per pound of N.

Table 3. Fertilizer costs for a variety of organic fertilizers. Prices are from Peaceful Valley and Seven Springs Farm.

Fertilizer Type	% N	Amount	Price	Shipping	Total Cost	Cost per Pound of N
			----------------------------$----------------------------			
Cyano-fertilizer*	0.003	75,000 gal	--	0	5373.41	89.56
Blood meal	12	5 lbs	14.99	11.99	26.98	44.97
Feather meal	12	6 lbs	11.99	11.99	23.98	39.97
Alfalfa meal	2.5	5 lbs	7.99	11.99	19.98	159.84
Fish emulsion						
Aqua Power	5	5 gal	49.99	24.91	74.90	33.63
Alaska Fish	5	5 gal	81.00	45.90	126.90	56.97

*The cyano-fertilizer costs are based on the 96 m^2 raceway size and a 5-yr period including construction and maintenance costs.

In addition to direct price comparisons per lb of N, on-site fertilizer production also provides intangible value and addresses farmer needs and preferences. In our market survey, farmers professed needs to reduce the bulkiness and handling costs of low nutrient materials, minimize odor, and allay sustainability concerns. Although, at this time, we cannot assign a hard dollar value to these advantages of cyanobacterial bio-fertilizer, they do play a role in farmer decision-making.

The current cost-benefit ratios for organic fertilizers range from 0.6 – 2.7 (Table 4). Therefore, the current production system already presents a cost savings when compared to alfalfa meal fertilizer. However, to be competitive with a wide range of organic fertilizer products, increasing productivity by 2-3 times, or reducing costs by ½ to $^{1}/_{3}$, or a combination of the two will be required.

Table 4. Current cost-benefit ratios comparing on-farm cyano-fertilizer with organic fertilizers from off-farm sources.

Fertilizer	Current Cost-Benefit Ratio
Blood meal	2.0
Feather meal	2.2
Alfalfa meal	0.6
Fish emulsion	
Aqua Power	2.7
Alaska Fish	1.6

Organic farmers using cyanobacterial bio-fertilizer to replace fish emulsion are predicted to break even in 1.6 years or less (Table 5). This conservative scenario is for a small farm (2 ha) using the cheapest source of fish emulsion. In most of the other scenarios, an organic farmer could break even on their fertilizer costs in less than 1 year. A larger farm will reach the break-even point more quickly due to the lower costs of raceway construction at scale.

Table 5. Time to breakeven for on-farm cyano-fertilizer production on organic farms of varying sizes. Assumptions: 9% of the farmland would be put into cyano-fertilizer production. Organic vegetable production requires 50 lb N/acre on average in addition to compost application.

Farm Size	Raceway Construction Costs ($\$/m^2$)	Time to Breakeven (years)	
		Minimum	Maximum
2 ha	10	0.9	1.6
10 ha	8	0.7	1.3
50 ha	5	0.5	0.8

Based on our on-farm experience applying cyanobacterial bio-fertilizer directly through drip and micro-sprinkler irrigation lines without any clogging, it is clear that applying the bio-fertilizer through fertigation is the most profitable scenario on farms with irrigation systems (nearly all vegetable farms in the western USA are dependent on irrigation).

In summary, cyanobacterial bio-fertilizer production can achieve breakeven periods of one to two years, which may be decreased by higher prices for competing fertilizers, additional income streams (e.g., carbon credits), or lower bio-fertilizer production costs.

Objective e. To impact farmer decision-making regarding fertilizer selection by sharing results through multiple methods

Activities are reported below under Outreach.

6. Conclusions and Discussion

Six of the seven organic fertilizers analyzed were found to contain auxin and salicylic acid; however, none of the fertilizers contained any measurable levels of cytokinins. The non-hydrolyzed fish emulsion contained a high auxin content and a low salicylic acid content, while the hydrolyzed fish emulsion was relatively high in salicylic acid and low in auxin. The cyano-fertilizer was high in both phytohormones.

In both the field-grown carrot study and the greenhouse pepper experiment, the cyano-fertilizer and the non-hydrolyzed fish emulsion treatments had the highest crop yields. This may be due to both of these fertilizers resulting in the largest auxin application rates.

There was no fertilizer effect on beta-carotene concentration in peppers; however, the non-hydrolyzed fish emulsion had higher beta-carotene concentration compared to other organic fertilizer treatments when applied to lettuce. In the lettuce study, the auxin application rates were positively correlated with the beta-carotene concentrations.

In the pepper study, fertilizer treatment affected phytohormone levels in pepper leaves at harvest time. Specifically, the non-hydrolyzed fish treatment had higher abscisic acid and 12-oxo-phytodienoic acid concentrations in the leaves.

In addition, both foliar seaweed products resulted in a significant reduction in the number of bell-shaped peppers harvested.

In the on-farm research carried out on organic peach orchards, application of cyano-fertilizer in addition to compost increased peach yield and reduced the growth of the tree trunks. In addition, cyano-fertilizer increased the SPAD chlorophyll readings of the leaves, and the SPAD readings were positively correlated to the distal leaf iron concentration.

The economic evaluation of the on-farm cyano-fertilizer production system found that cyano-fertilizer is already competitive with the most expensive organic N fertilizers. However, to be competitive with fish emulsions and blood and feather meals, the cost per lb of N must be reduced to about half of current costs. Based on smaller-scale experiments with supplemental CO_2, it is likely that we will be able to double N fixation while increasing costs by only about 20%. We will be evaluating this approach in field-scale raceways during the summer of 2016.

7. Useful Tools, Information, and Resources for Farmers

We have written a manual entitled "Building a Raceway for Cyanobacterial Bio-fertilizer Production." Through the WSARE project funding described under Leveraged Resources below, a variety of additional tools will be developed to maximize our potential to reach a large number of farmers. We will develop a Cyanobacterial Bio-Fertilizer Production Manual that builds on the previous document. An extension fact sheet on Cyanobacterial Bio-fertilizer Utilization will also be written focusing on the best ways to use the bio-fertilizer to optimize crop yields and nitrogen use efficiency.

Furthermore, we will develop a short video on On-farm Production and Utilization of Cyanobacterial Bio-fertilizer that can be viewed online and at conferences. The video will serve as a way to introduce farmers to our work and to draw them to the more detailed documents available online.

8. Outreach

We extended project impact through a variety of conference presentations throughout the region (in order by date):

- 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. Developing an Organic On-Farm Bio-fertilizer Production System using Cyanobacteria, Organicology, Portland, OR, February 6-7, 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.*, 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.
- 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.
- 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, 2015; 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, 2015; 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, 2015; 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, 2015; Reno, NV.
- Davis, J.G. 2015. Challenges and Opportunities in On-Farm Cyano-Fertilizer Production and Utilization. Texas A&M University at Corpus Christi, Corpus Christi, TX, May 13, 2015.
- Davis, J.G. 2015. Challenges and Opportunities in On-Farm Cyano-Fertilizer Production and Utilization. Texas A&M University at Weslaco, Weslaco, TX, May 14, 2015.
- Davis, J.G.*, H. Storteboom, and M.S. Massey. 2015. Developing a solar-powered, on-farm fertilizer production system using cyanobacteria. SusTech2015 IEEE Conference on Technologies for Sustainability; July 30-Aug 1 2015 in Ogden, UT.

- Davis, J.G. 2016. On-farm Production and Use of Algae as Fertilizer, New Mexico Organic Farming Conference, Albuquerque, NM, February 19-20, 2016.
- Davis, J.G. 2016. Soil Fertility Management in Irrigated Organic Cropping Systems, High Plains Organic Farming Conference, Cheyenne, WY, February 24, 2016.
- Erwiha, G., A. Sukor, J. Ham, and J. G. Davis. 2016. Effect of organic nitrogen fertilizer source, application method, and application rate on ammonia volatilization from drip irrigated lettuce. Great Plains Soil Fertility Conference, Denver, CO, March 1-2, 2016.
- Jama, A., and J.G. Davis. 2016. Nitrogen mineralization of *Azolla mexicana* applied as fertilizer compared to compost and cyanobacterial fertilizers. Great Plains Soil Fertility Conference, Denver, CO, March 1-2, 2016.
- Widiastuti, D.P., and J.G. Davis. 2016. Optimization of the nutrient media for *Azolla mexicana* growth for use as fertilizer. Great Plains Soil Fertility Conference, Denver, CO, March 1-2, 2016.

9. Financial Accounting

Table 6. Budgeted and actual expenses of this OFRF-funded project in US Dollars.

	Budgeted ($)	Actual ($)
Graduate Research Assistant	8,190	8,233
Fringe Benefits	407	659
Materials and Supplies	1,963	15
Other Direct Costs	3,440	5,093
TOTAL	14,000	14,000

The Other Direct Costs were for laboratory analyses of β-carotene (in pepper and lettuce studies) and phytohormones (in fertilizers in all of the studies and in leaves of the pepper study). These analyses were more expensive than expected, and we were able to cover most field supplies from the USDA Western Sustainable Agriculture Research and Education project described below under Leveraged Resources.

10. Leveraged Resources

We received a grant of $293,599 from the USDA Western Sustainable Agriculture Research and Education (WSARE) Program that covered the costs of most of the field work described in this proposal. This funding allowed us to use OFRF funding specifically for the beta-carotene and phytohormone components of our research. The WSARE project runs through 2017 and will also fund development of educational resources for farmers.

In addition, we received $10,000 from the CSU Water Center which went towards optimization of the cyano-fertilizer production system described under Objective d in the Project Results section above.

Colleagues at the University of Wyoming led by Dr. Jay Norton became interested in our work and have received a Wyoming Specialty Crops grant for 2016-2017 to evaluate cyano-fertilizer production and utilization on a diversified vegetable farm near Cody, WY.

11. References

Abadia J., and A. Abadia. 1993. Iron and plant pigments p. 327-343. In: L.L. Barton and L. Hemming. Iron chelation in plants and soil microorganisms. Academic Press Inc., New York.

Belkhodja, R., F. Morales, M. Sanz, A. Abadia, and J. Abadia. 1998. Iron deficiency in peach trees: effects on leaf chlorophyll and nutrient concentrations in flower and leaves. Plant and Soil 203:257-268.

Edlund, A., S. Eklof, B. Sundberg, T. Moritz, and G. Sandberg. 1995. A microscale technique for gas chromatography-mass spectrometry measurements of pictogram amounts of indole-3 acetic acid in plant tissues. Plant Physiol. 108:1043-1047.

Pan, X., R. Welti, and X. Wang. 2010. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. Nature Proto. 5:986-992.

Wilhelm S.W., and Trick, C.G. 1994. Iron-limited growth of cyanobacteria: multiple siderophore production is a common response. Limnology and Oceanography. 39(8): 1979-1984.

12. Photos and Other Addenda

Photos were emailed to OFRF already and are, therefore, not included here.

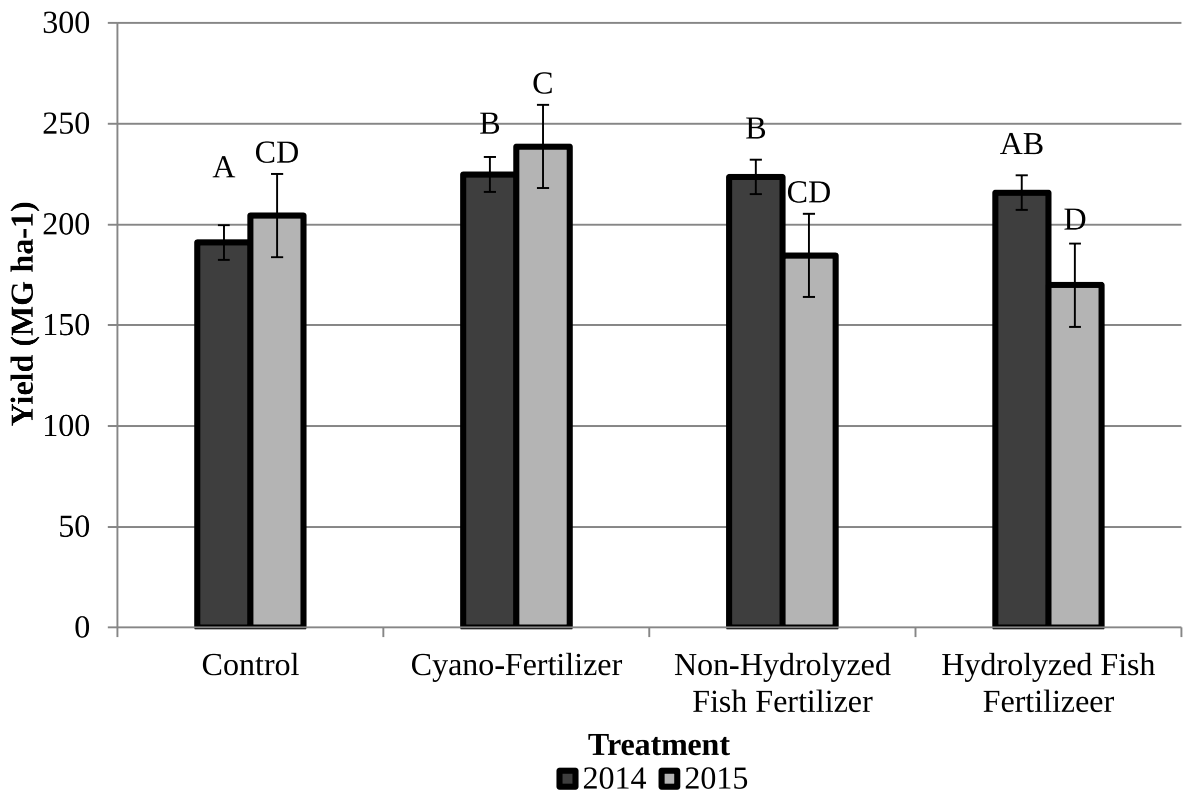

Figure 1. Average yield of carrots in MT ha^{-1} grown in a field experiment in Fort Collins in 2014 and 2015. Treatments that share a common letter are statistically similar. Treatments with different capital letters are statistically different from one another ($p<0.05$).

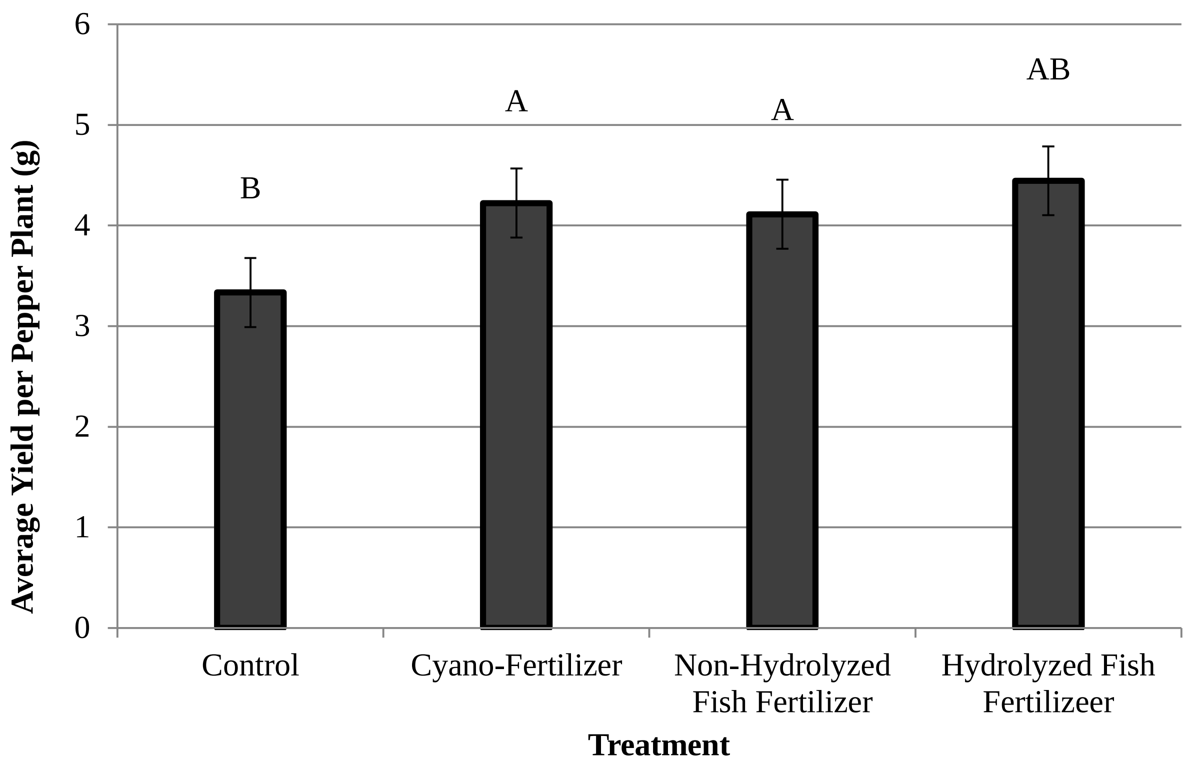

Figure 2. Average yield per plant (g) from bell peppers grown in a greenhouse experiment in Fort Collins, CO in 2015. Treatments that share a common letter are statistically similar. Treatments with different capital letters are statistically different from one another ($p<0.05$).

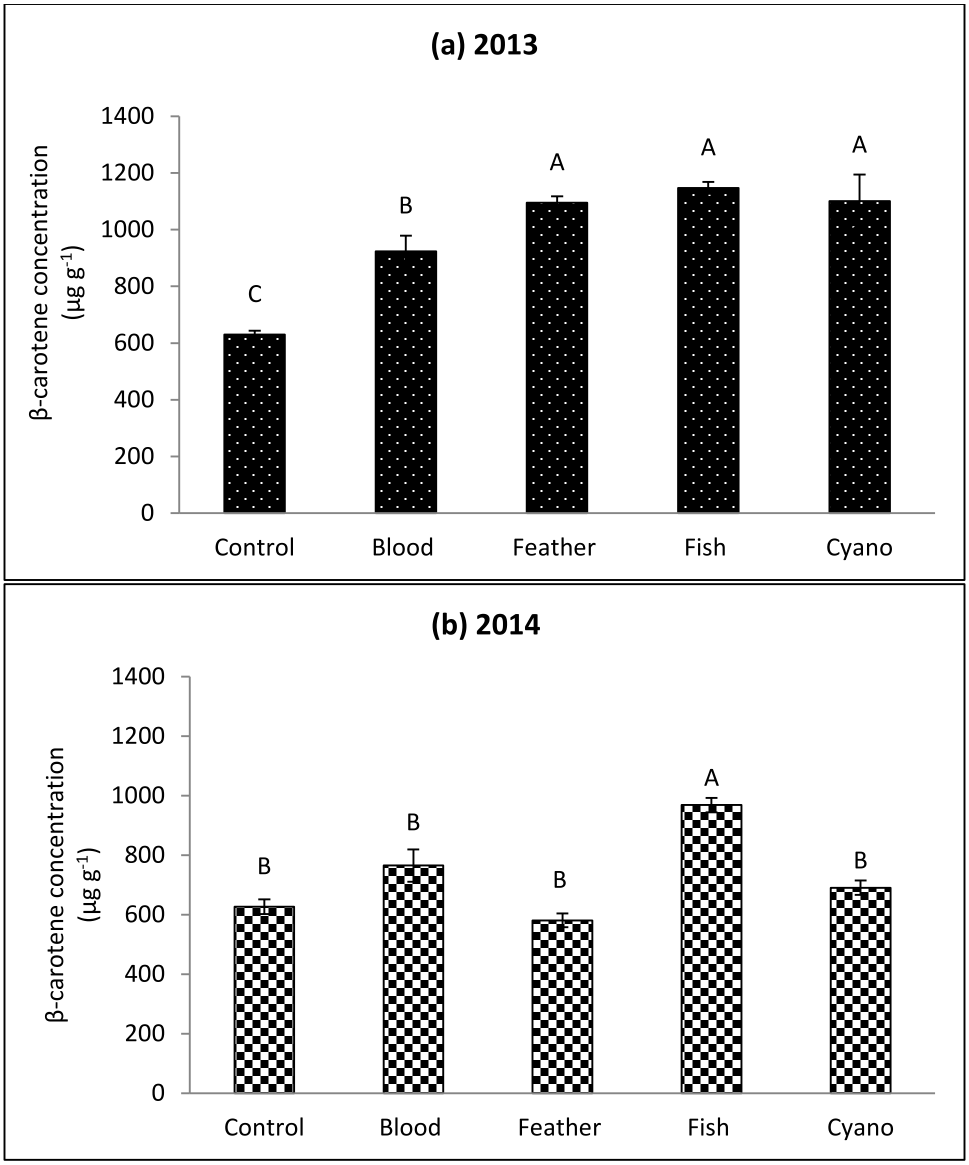

Figure 3. Effects of different organic N fertilizers on β-carotene concentration of drip-irrigated lettuce. Fertilizers were applied at 56 kg N ha^{-1}. Means with the same letter are not significantly different at $P < 0.1$ using Tukey's Studentized Range (HSD) test of mean separation. Bars represent standard errors of mean. Blood = Blood meal; Feather = Feather meal; Fish = Non-hydrolyzed fish emulsion; Cyano = Cyano-fertilizer.

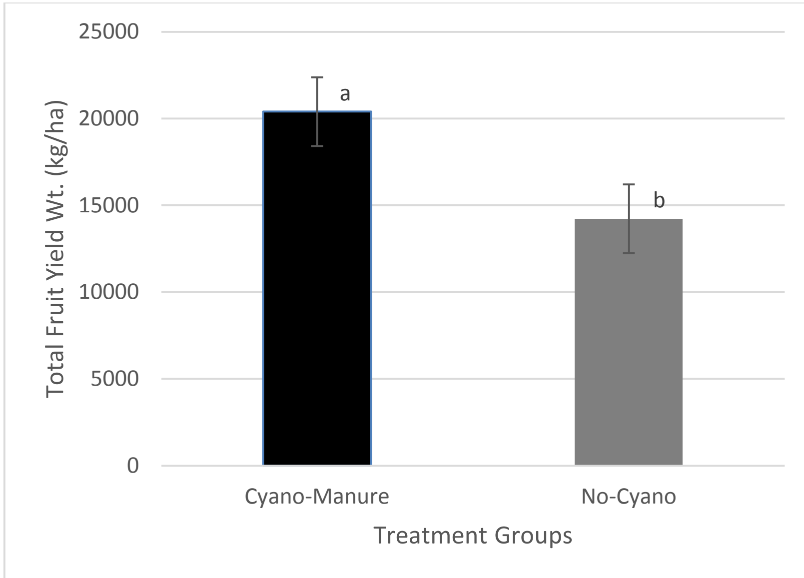

Fig. 4. Comparison of the 2014 average peach yield of plots at Farm B, in the 2014 fertilization section, of both treatments which included Cyano-Manure and No-Cyano. Means without a common letter are different, as determined by ANOVA (*P≤0.05*), and error bars represent standard error.

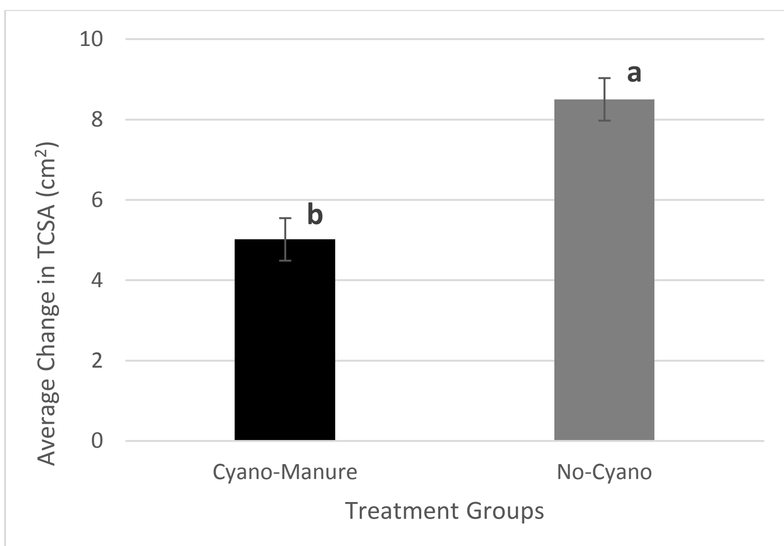

Fig. 5. Comparison of the average growth in trunk cross sectional area of peach trees treated with Cyano-Manure and No-Cyano, on Farm B in 2014. Means without a common letter are different, as determined by ANOVA (P≤0.05), and error bars represent standard error.