THESIS

EVALUATION OF CYANOBACTERIAL BIOFERTILIZER AS A SUPPLEMENTAL OR SOLITARY FERTILIZER ON PEACH YIELDS, LEAF TISSUE NUTRIENT CONCENTRATION, AND TRUNK GROWTH

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ABSTRACT

EVALUATION OF CYANOBACTERIAL BIOFERTILIZER AS A SUPPLEMENTAL OR SOLITARY FERTILIZER ON PEACH YIELDS, LEAF TISSUE NUTRIENT CONCENTRATION, AND TRUNK GROWTH

Nitrogen (N) is the nutrient applied in the greatest quantities to peach trees and is a necessary component of proteins. As a result, carbon assimilation is dependent upon adequate levels of N in leaf tissue. Cyanobacteria are a type of bacteria which can fix gaseous N from the atmosphere enzymatically. This N fixation can be exploited in a cyanobacterial biofertilizer (cyano-fertilizer) production raceway, which allows farmers to grow their own source of N with relatively small energy inputs. Cyano-fertilizer was grown on three peach farms in Western Colorado, and applied to peach orchards in combination with a chicken feather meal (mixed with meat and bone meal), a dried chicken manure, and separately in comparison to a conventional foliar fertilizer, fish emulsion fertilizer foliarly applied, and a soil application of fish emulsion fertilizer. Treatments were assigned to experimental units across three separate farms (Farms A, B, and C) and arranged using Randomized Complete Block Designs.

Peach fruit yield, trunk cross sectional area, leaf tissue nutrient concentrations, soil nutrient concentrations, SPAD and fruit juice quality characteristics were measured. A significant fruit yield increase was seen on Farm B in treatments which included cyano-fertilizer and manure (Cyano-Manure), versus manure alone (No-Cyano). Trunk cross sectional area showed less growth in treatments including cyanobacteria on Farm B. Significantly higher leaf tissue S, P, and Cu concentrations were found in Cyano-Manure treatments on Farm B; however,

significantly greater Ca concentrations were found in the No-Cyano treatment. Chlorosis was present throughout Farm B and so relative leaf chlorophyll content was estimated by measuring Soil Plant Analysis Development (SPAD). SPAD readings were positively correlated with leaf Fe concentration. In the 2015 fertilization section, SPAD readings were higher in Cyano-Manure treatments despite the relatively low amount of Fe present in the cyano-fertilizer, suggesting that cyano-fertilizer may have increased Fe uptake by the trees.

Significant differences in leaf micronutrient concentrations were found among treatments in Farm C. Across all farms, treatment effects were masked by three unforeseen events. First, a large infestation of aphids on Farm A caused the death of young vegetative tissue and also killed young peach fruit. Second, a freezing event during bloom, killed most of the fruit on two of the farms. Lastly, there were prior fertilizations earlier in the season on Farm C which lowered the impact additional fertilizer had on the trees.

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INTRODUCTION

Nitrogen (N) is the most commonly limiting nutrient in plants. Nitrogen fertilization of peach trees results in higher total fruit yield and larger individual fruit as a result of delayed maturation (Rader et al., 1985; Saenz et al., 1997). However, Taylor and van den Ende (1969) did not find evidence that the stored N content of the trees influenced numbers of flowers or fruit, because there was a prioritization of reproductive growth over vegetative growth, and the amount of stored N was evidently high enough to meet the reproductive demand for N, given the fixed amount of fruiting sites provided by the previous season's growth, having received 0.56 kg N/tree per year for the previous three years. Conversely, Cain and Meilenbacher (1956) did find increases in total fruit yield weight and fruit count in trees which received high N fertilization (0.24 kg N/tree) compared to low N fertilization (0.079 kg N/tree), and this was attributed to an increase in vegetative growth, creating more potential fruiting sites in the year after fertilization. Trunk growth has also been found to increase with N fertilization in the year after fertilization (Cain and Meilenbacher, 1956).

Carbon (C) assimilation is highly correlated with the N content of peach leaves (DeJong, 1982), and N fertilization increases C assimilation in peach trees by increasing the photosynthetic capacity of partially shaded leaves (DeJong et al., 1989). Taylor (1966) suggested that during the period after shoot growth stops, trees begin to store N to be used for new growth in the following spring, and that the amount of N stored is proportional to the amount of N available to the tree. Both the leaf N content and shoot growth were also proportional to the amount of N stored in the woody tissues of the tree from the previous season (Taylor and van den Ende, 1969).

Much research has been done to determine whether the proper timing of N fertilization of stone fruit trees should be spring or autumn or a combination of the two (Bi et al., 2003; Jordan, 2013; Taylor and May, 1967). These researchers have shown that trees, especially mature trees, have the ability to buffer their N supply with internal stores of N, rather than being completely dependent on a specifically timed fertilization. Trees which received 200 kg N/ha compared to non-fertilized trees contained 215% higher storage N content while dormant, totaling 0.11 kg/tree or 58.7% of the applied N rate being stored in the woody tissue of the trees, suggesting that a significant portion of the N budget is stored within the trees (Niederholzer et al., 2001). Fifty percent of leaf N is mobilized and stored in woody tissue during leaf senescence (Niederholzer et al., 2001). In organic systems, fertilization timing is even more complicated as plant available N is released slowly throughout the year (Gutser et al., 2005), as opposed to conventional N fertilizers which tend to be plant available at the time of application. In organic farming systems, N comes from manure, compost, and animal by-products such as fish emulsions, which usually require movement of large amounts of material from off-farm locations, or N fixation by legumes. Conventional farmers are able to get N from synthesized ammonia by way of the Haber-Bosch process (Erisman et al., 2008) which requires great amounts of energy to produce and transport. Conventional peach farmers in the Grand Valley of Colorado often apply N fertilizers foliarly, as N fertilizers such as urea are easily absorbed through leaves and translocated through trees (Rosecrance et al., 1998).

Anabaena spp. and other types of cyanobacteria use nitrogenase to fix gaseous N from atmospheric N_2 gas, by way of a type of specialized cell called heterocysts (Fay, 1992; Gallon, 1992; Thiel and Pratte, 2001). Cyanobacteria can be grown on-farm using organic nutrient media (Barminski et al., 2016; Benemann, 1979), and has been shown to be an effective organic

fertilizer in annual vegetable crops (Sukor, 2013; Yoder, 2014). Prior research has shown that cyanobacterial biofertilizer (cyano-fertilizer) can lead to higher yields than composted steer manure in lettuce (Sukor, 2013). Cyano-fertilizer has been shown to decrease soil pH and increase β-carotene concentration in kale (Davis et al., 2013). The decrease in soil pH also may have led to the increased uptake of Zn and Fe in kale (Davis et al., 2013). On-farm production of N fertilizer can decrease the energy involved in transporting fertilizers from off-farm locations.

Along with N, other nutrients such as iron (Fe) are important for peach production. Iron chlorosis is a decrease in the amount of chlorophyll within a leaf resulting from an Fe deficiency (Abadia and Abadia, 1993). Moderate Fe chlorosis has been shown to decrease peach fruit yield weights by up to 83% (Alvarez-Fernandez et al., 2011). In addition, Fe chlorosis has been known to lead to higher K concentrations in leaf tissue (Abadia et al., 1985; Belkhodja et al., 1998). Fe deficiency also can lead to variable fruit size and cause changes in fruit pH, fruit total soluble solids (TSS), and vitamin C content (Alvarez-Fernandez et al., 2011). Soil Plant Analysis Development (SPAD) is a measurement which has been used on peach leaves, to estimate leaf chlorophyll content per leaf area and to quantify the extent of chlorosis (Alvarez-Fernandez et al., 2011; Belkhodja et al., 1998). Cyanobacteria are known to release siderophores in Fe limiting environments which chelate Fe into a bio-available form (Wilhelm and Trick, 1994).

The objective of this research was to evaluate the effectiveness of cyano-fertilizer as both a supplemental N fertilizer source in conjunction with chicken manure compost, and as a solitary fertilizer in comparison to both conventional and organic options. The apparent effect of the cyano-fertilizer upon Fe chlorosis was also investigated to determine whether the effect was caused by increased Fe concentration in leaf tissue or another possible factor such as leaf S concentration. We hypothesized that cyano-fertilizer application would lead to equal or greater

yield and vegetative growth in peach in comparison to no cyano-fertilizer application. We also hypothesized that cyano-fertilizer application would result in peach leaf tissue nutrient concentrations, specifically N, P, and Fe, which are equal to or greater than the other fertilizer treatments, and as a result, the occurrence and severity of chlorosis would be reduced.

MATERIALS AND METHODS

Cyano-Fertilizer Production and Application

In 2014 and 2015 cyanobacteria (*Anabaena* spp.) were grown at two organic fruit orchards (Farms A and B) in Hotchkiss, CO, and one conventional farm the Colorado State University Research Center in Orchard Mesa, CO (Farm C). The cyanobacteria were grown in paddlewheel-agitated raceway ponds of 2,366 liters to 4,921 liters in volume, using an organic nutrient media (Barminski et al, 2016). Nutrients were added to the raceway individually for NaCl, KCl, and $MgSO_4$-$7H_2O$, while $FeSO_4$-$7H_2O$ was first mixed with distilled water before adding, and the other micronutrients: MnSO4H_2O, Na_2Mo4- $2H_2O$ (99% purity), $ZnSO_4$-$7H_2O$, $CuSO_4$-$5H_2O$, H_3BO_3 and $CoCO_3$, were added together. One nutrient was added to the raceway every 2-5 minutes with the paddlewheel circulating the water the entire time. The cyanobacteria were then applied directly to the field as a biofertilizer after a growth period of 12 to 18 days, when the cyanobacteria culture was healthy. Depending on the farm and season, 3 to 9 applications were made to the orchards per season, dependent on success of culture survival and nitrogen fixation (Table 1). During the growing period, parameters of the biofertilizer were analyzed using a handheld pH meter (Hanna Instruments, Woonsocket, RI) to measure both pH and temperature between 12:30 pm and 1:00 pm during peak photosynthesis. The N concentration was measured during each application using a Hach DR 3900 spectrophotometer (Hach Co., Loveland, CO).

The application of the biofertilizer was made using a sump pump connected to an irrigation system which mimicked the systems used at the farms. At farms A and B this was a microsprinkler system, and at farm C this was a drip irrigation system. The biofertilizer was pumped either directly from the raceway pond, or from a 946 liter tank which was taken to the application site directly after being filled from raceway pond. Volume applied was recorded and

multiplied by N concentration to determine the amount of N applied. Throughout an application, roughly 90 percent of a raceway pond would be applied. Tap water and a proportionate amount of nutrient media were then added to the pond to start a new growing period.

Plot Layout and Treatments (Farms A and B)

At Farms A and B the peach cultivar used was Sun Crest grown on a Lovell rootstock. The spacing at Farms A and B was 1.9m x 4.6m and 1.6m x 4.6m, respectively. The trees at Farms A and B were planted in 2008 and 1999 respectively. In 2014 Farm A was in the second year of transitioning to organic production, and the Farm B orchard had been organic since it was planted. The soil type at Farm A was an Agua Fria Loam which is classified as mesic Calcic Paleargids, and Farm B had a Mesa Loam soil which is classified as mesic Typic Caliargids (Natural Resources Conservation Service, 2008a; Natural Resources Conservation Service, 2008b).

For farms A and B, 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. Trees of the same apparent health, meaning similar size and no apparent deficiencies or obvious disease symptoms, were arranged using a Randomized Complete Block Design (RCBD) with five replications per treatment. At farm A, two treatments were applied. The first treatment, "Chicken Meal," was 112 kg N/ha 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 (Table 2). A second treatment, "Chicken Meal+Cyano," was 112 kg N/ha True Organic 12-3-0 with 11.4 kg N/ha from cyano-fertilizer (Table 2). 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. A freezing event on April 3, 2015 killed most of the blossoms on the trees, and it was decided to move the experiment to a site that was likely to have a full crop load, rather than continue the experiment.

At farm B, three treatments were applied: 1) "High Manure" was 112 kg N/ha from Richlawn 5-3-2 (Platteville, CO) a dried poultry manure, 2) "High Manure+Cyano" was 112 kg N/ha from Richlawn 5-3-2 with 4.9 kg N/ha from cyano-fertilizer, and 3) "Low Manure+Cyano" was 84 kg N/ha from Richlawn 5-3-2 with 4.9 kg N/ha from cyano-fertilizer (Table 3). In addition to N, other nutrients were applied with the cyano-fertilizer which originated with the growing medium used (Table 4). The cyano-fertilizer N rate of the treatments on farms A and B differed because the rate of application was dependent upon the rate of N fixation by the cyanobacteria. 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 (Table 5). 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 (Table 3). April 3, 2015 a freezing event killed most of the blossoms on the trees in the orchard, resulting in roughly 65% yield weight and fruit count reductions, since temperatures dropped to approximately -3.8 degrees C for a period of at least 3 hours (Colorado State University, 2015).

Plot Layout and Treatments (Farm C)

In 2015 an experiment was started at the Western Colorado Research Center in Orchard Mesa, CO (Farm C), rather than continuing the experiment at Farm A, because of the extensive freeze damage on April 3, 2015 at Farm A, in Hotchkiss, CO. At Farm C, the cultivar was Crest Haven. The spacing at Farm C was 4.0 meters between trees and 4.9 meters between rows of

trees. The soil series for Farm C was Turley, a fine-loamy mixed, active calcareous soil, classified as mesic Typic Torriorthents (Natural Resources Conservation Service, 2008c). On Farm C, experimental plots consisted of 2 adjacent trees. The four treatments on Farm C included: 1) "Cyano," a cyano-fertilizer treatment which was applied by drip line, 2) "Conventional Foliar," Pro-Sol 20-20-20 Turf fertilizer (Ozark, AL) foliarly applied, 3) "Fish Foliar" Alaska (Walnut Creek, CA) fish emulsion fertilizer (5-1-1) was foliarly applied and another treatment, 4) "Fish Drench," with the Alaska fish emulsion fertilizer applied directly to soil (Table 6). A total of four applications of fertilizer were made during the experimental phase (from May 2015 – August 2015), applying a total of .021 kg of N per tree (10.8 kg N/ha) with each of the other treatments matching the amount of N applied in the cyano-fertilizer applications. The treatments were replicated 6 times each except for the Fish Foliar treatment, which was only replicated 5 times, because of a lack of suitable trees. Therefore the experimental design was an almost complete Randomized Block Design. Both applications of fish-based fertilizer and conventional fertilizer were applied via a powered hand sprayer (diluted to a concentration of .0013 kg N per L). The volume applied was regulated by the amount of time the sprayer was engaged, the rate of which was determined beforehand by timing length of time needed to spray 1.9 L. After the experiment was in progress, it was discovered that in April all of the trees in the orchard had received 2 applications of Pro-Sol, at a rate of 2.24 kg N/ha each, totaling .005 kg N applied per tree, in addition to other nutrients included within the fertilizer (Table 7).

Measurements (Farms A and B)

In summer of 2014, length of first year branches was measured. Branches were selected which were initially of similar size, with one branch being selected from each compass

directional quadrant at a height of 1.5 to 1.8 meters. Measurements were taken from the branch crotch to the growing tip at the apical meristem. This measurement was not continued in 2015 because the variability in branch growth was extremely high.

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 for both farms in 2014, but just Farm B in 2015. Circumferences were measured at a height of 21.3 cm from the orchard floor. Circumferences were then converted into TCSA by the formula: TCSA= (Trunk circumference/2π)^2 x π. TCSA change was calculated by the formula: TCSA change= (End of season TCSA- Beginning of season TCSA).

Sampling for midseason leaf tissue nutrient analysis was done following the protocol given by A & L West Labs (www.al-labs-west.com). Leaf sampling date at Farm A was July 14, 2014; at Farm B the sampling dates were July 14, 2014 and July 23, 2015. Two leaves per compass directional quadrant were selected from each measured tree at a height of 1.2 to 1.8 meters from the orchard floor, from mid shoot of first year shoots, with petioles attached, and were not washed before sending to analysis labs. Leaf tissue samples were then analyzed by A & L Western Laboratories (Modesto, CA) in 2014 using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis using the NAPT (North American Proficiency Testing) methods P 2.20 for N and P 4.30 for P, S, K, Mg, Ca, Na, Fe, Al, Mn, B, Cu and Zn (Gavlak et al., 2005). In 2015 Ward Laboratories was used for analysis (Kearney, NE). Leaf N concentration was analyzed by combustion of leaf tissue at 1050° C, and N was quantified by thermal conductivity using a LECO TruMac Nitrogen Combustion Analyzer (Miller et al.,1997). Leaf Zn, Fe, Mn, Cu, Na, P, S and Mo were determined by digesting the sample in nitric acid, hydrochloric acid, and hydrogen peroxide and then analyzing the sample in an ICAP (Inductively

Coupled Argon Plasma) (Campbell and Plank, 1991). At Farm B in 2015, leaf tissue samples were taken a second time using a modified protocol taking fully expanded leaves from first year branches from 10-15 centimeters from the growing tip to sample for immobile micronutrient deficiencies on August 14.

On April 26, 2014, April 25, 2015, November 28, 2014 and October 24, 2015, soil samples were taken from four locations within each plot to a depth of 15 cm, to determine the early season and late season soil nutrient concentrations (Table A1; Table A2; Table A3). Soil cores were combined in a plastic bag, air dried, ground, sieved through a 2 mm sieve, and analyzed by Ward Laboratories. Soil pH was determined by creating a 1:1 soil to water solution and measuring the supernatant with a pH meter (Mc Lean, 1982). NO_3^- N and SO_4^{2-} S were extracted in a calcium phosphate solution, and measured using a Lachat FIA (Flow Injection Anaylsis) Analyzer (Loveland, CO) (Combs et al., 1998; Geldemen et al., 1998). Soil Zn, Fe, Mn, and Cu, were extracted using DTPA to chelate the nutrients and analyzed with ICAP (Whitney, 1998). Soil P was extracted using the Mehlich 3 method and analyzed using a Lachat QuikChem (Loveland, CO). Soil organic matter was estimated by loss on ignition (Combs and Nathan, 1998). Comparisons were then made based on the change in the soil properties before and after the growing season.

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 A peaches were harvested on August 28, 2014 with all peach fruit being harvested by David Sterle personally. 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.

A six-peach sample was taken from each plot for in-lab peach juice analysis in both 2014 and 2015. Two vertical slices were taken from each peach 90 degrees from the suture of the peach. Peach slices were juiced, and the juice was gravity fed through cheese cloth into a test tube. Juice pH was measured, and soluble solids content (SSC or °Bx) of juice was measured using an Atago PR-101 Refractometer (Bellevue, WA). The ratio of sugars to acidity (SSC:$[H^+]$)was estimated by transforming pH into a measure of acidity which increased in number with increasing acidity through the formula: 7-$[H^+]$, as a substitute for the commonly referenced ratio SSC: Titratable Acidity (TA) ratio (Daane et al., 1995; Olienyk et al., 1997).

Measurements (Farm C)

TCSA measurements, soil sampling (5/30/15; 10/24/15; Table A4), leaf tissue analysis (7/24/2015), and peach juice analysis were all done using the same methods described above for Farms A and B. Fruit was harvested (8/25/15) by staff at the Western Colorado Research Center.

Statistical Analysis

The data for each farm was analyzed using a Randomized Complete Block Design (RCBD). Data analysis was done using SAS 9.4 (SAS Institute Inc., Gary, NC). The Mixed procedure was used to perform Analysis of Variance (ANOVA), and orthogonal contrasts were

evaluated using the least squares mean estimates from the lsmestimate option, by combining treatments which included cyano-fertilizer and comparing them to the treatment without cyano-fertilizer. Differences between basal leaf tissue nutrient concentration means were evaluated, and changes in soil nutrient concentrations at Farm C evaluated using Duncan's multiple range test. Correlations between yield, SPAD, TCSA change, and leaf tissue nutrients were analyzed using the Corr procedure. Due to high variability in data caused by differences in peach fruit thinning, branch pruning and tree health, an alpha value of 0.1 was used as a critical significance level, and p-values below 0.1 but above 0.05 will be referred to as "marginally significant."

RESULTS

Blocking Effects

Statistical analysis showed that blocking effects were significant for some response variables across all farms, while not being significant across most response variables. The blocking effects were significant for fruit yield, leaf P concentration, and leaf K concentration for Farm A. Blocking effects were significant for P:Fe ratio in basal leaves, fruit juice pH, TCSA growth, and leaf Cu concentration on Farm B in the 2014 fertilization section and fruit yield, fruit juice pH, and leaf Ca concentration in the 2015 fertilization section. On Farm C, blocking effects were significant for basal leaf P concentration, basal leaf Mg concentration, leaf Zn concentration, and changes in the amount of soil Ca and Mg from the beginning to the end of the season. Although not significant for most of the response variables, blocking was still included in ANOVAs because not including blocks did not appear to affect the occurrence of significant differences among treatments.

Farm A

There were no significant differences among treatments in yield, fruit count, TCSA growth, leaf tissue nutrient concentrations (Table 8), fruit juice pH, SSC, or SSC: $[H^+]$ ratio. Higher fruit count was positively correlated to leaf N ($R=0.805$; $P=0.005$; data not shown). Yields were highly variable due to aphid infestation, which masked treatment effects (Figure 1; Figure 2).

Farm B 2014 Fertilization Section

In 2014 fruit yield for Farm B was significantly higher in plots treated with both a cyano-fertilizer and manure (Cyano-Manure) compared to manure (No-Cyano) the manure treatment alone (P=0.0346; Figure 3), whereas there was no significant difference found among individual treatments. The same was true for average fruit count, which was significantly higher (P=0.0216; data not shown) in Cyano-Manure compared to No-Cyano groups, and marginally different (P=0.0781; data not shown) between individual treatments, High Manure and High Manure+Cyano. In 2015 there were no significant differences found in fruit yield or average fruit count, among treatments or between treatment groups.

In 2014 TCSA growth was higher in No-Cyano than Cyano-Manure (Figure 4), and versus both treatments High Manure+Cyano and Low Manure+Cyano (P= 0.0042 and 0.0004, respectively; data not shown). No differences in TCSA growth were found among any treatments in 2015 as residual effects of the 2014 cyano-fertilizer fertilization.

In 2014 no differences were found in basal leaf tissue nutrient concentration; however, means tended to be higher in Cyano-Manure treatments than in No-Cyano treatment (Table 9). In 2015 marginally significantly higher basal leaf tissue S (P=0.0882) and Cu (P=0.0868; data not shown) concentrations were found in Cyano-Manure treatments than in the No-Cyano treatment, and marginally significantly higher distal leaf tissue P (P=0.0945) was observed in Cyano-Manure treatments than No-Cyano using contrasts (Table 9), but among separate treatments these differences were not seen. Additionally, both basal and distal leaf tissue nutrients tended to be higher in Cyano-Manure treatments compared to the No-Cyano treatment (Table 9). End of season soil NO_3-N levels were positively correlated to basal leaf N levels the following year (R=0.630; P=0.0119; data not shown).

In 2015 no differences were found in SPAD readings among treatments. SPAD was significantly and positively correlated to distal leaf P concentrations (R=0.643; P=0.0097; data not shown).

In 2014 no difference in fruit juice pH or fruit SSC was found among treatments. In 2015 fruit juice pH was significantly lower in Cyano-Manure treatments (Figure 5); however, the SSC:[H] ratio was the same between treatment groups (Figure 6).

Farm B 2015 Fertilization Section

There were no significant differences in fruit yield (Figure 7) or TCSA growth among treatments or between treatment groups.

Marginally significant differences were found in Basal leaf tissue Ca (P=0.0600; Table 10) and B (P=0.0854; data not shown) concentrations being higher in the No-Cyano treatment, than in Cyano-Manure treatments, but this difference was not evident among individual treatments. No other significant differences were seen among treatments or between treatment groups for basal leaf nutrient concentrations. Distal leaf S concentration was significantly higher (P=0.0388) in Cyano-Manure treatments compared to No-Cyano treatments, but not significantly different for any other nutrient (Table 9) or among separate treatments (data not shown).

SPAD readings were significantly higher (P=0.0350) in Cyano-Manure treatments compared to the No-Cyano treatment (Figure 8), but not different among separate treatments. SPAD was significantly and positively correlated to the SSC:[H^+] ratio (R=0.789; P=0.0009; Table 11), cyano-fertilizer N amount applied (R=0.571; P=0.0261; Table 11), and distal leaf Fe concentration (R=0.571, P=0.0136; Table 11). Distal Leaf K concentrations were negatively correlated to SPAD readings (R=-0.662, P=0.0072; Table 11). Significant correlations were

found between SPAD and P/Fe ratio (R=0.594, P=0.0196; Table 11) and K/Ca ratio (R=0.493, P=0.062; Table 11) in distal leaves. There were no differences seen in the change, over the course of the season, in soil organic matter, pH, N, P, K, S, Ca, Mg, Fe, Mn, Zn, or Cu, among treatments.

Farm C

In 2015 no significant differences in yield were found among treatments (Figure 9), fruit count (data not shown), or TCSA growth (data not shown). A marginally significant difference in basal leaf Cu concentration (P=0.0995) was found between the Conventional Foliar treatment and Fish Drench (data not shown). The Fish Foliar treatment was higher in leaf Fe concentration than the Fish Drench treatment (Table 12). Leaf Zn concentration was higher in Fish Drench than in the Cyano treatment and Fish Foliar treatment (Table 12).

No significant differences were found among treatments in average fruit juice pH or SSC. There was a significant positive correlation found between SSC:$[H^+]$ ratio and leaf Fe concentration (R=0.43; P=0.0391; data not shown).

DISCUSSION

Farm A and B Fruit Yield and Growth

At Farm A in 2014, the data was highly variable due to a large amount of aphids on over half of the experimental area. The aphids caused death or stunting of first-year branches, and severely limited the fruit set of the trees. Fruit set heterogeneity was high (Figure 1), with one fruit per tree on several trees, while another plot had an average of over 100 fruit per tree. As a result no difference in fruit yield was seen between treatments (Figure 2). This effect probably masked treatment effects because it increased the overall variation in the orchard. Although nutrient exportation was limited by the low investment in fruit as nutrient sinks, it is likely that trees with damaged first-year branches would have low yields in the subsequent year as well, since peach fruits only grow on second-year branches. Also, no correlation was seen between TCSA growth and fruit set (data not shown), perhaps indicating that the damage done by the pests was sufficient to prevent trees from producing extra vegetative growth. More vigorous vegetative growth would be expected because the trees did not have to allocate resources to fruit, allowing for greater investment in vegetative growth.

At Farm A, higher fruit count was strongly associated with higher levels of leaf N. While it is not unusual to find higher leaf N levels associated with higher fruit count (Johnson, 2008), since the aphid pests were clearly the reason for lower fruit count, it suggests that this correlation must be due to other factors. There are many possible reasons for the positive correlation between fruit count per tree and high leaf N under these circumstances. First of all, there may have been a significant amount of N lost directly to the pests. Second, trees may have been more susceptible to pest damage at lower leaf N concentrations although Daane et al. (1995) found the

opposite to be true in regard to moth pests in peaches. Finally, trees may have mobilized some N away from sensitive new growth in response to the pest threat.

For Farm B in 2014, the Cyano-Manure treatments yielded significantly higher in terms of total fruit weight (Figure 3) and fruit count (data not shown) than the No-Cyano treatment. The No-Cyano treatment had lower yields, but greater TCSA growth, indicating that lower fruit set may contribute to greater vegetative growth (Figure 4). Cyano-Manure treatments did receive roughly 11% more water than the No-Cyano treatment through the treatment period (May 28-August 1, 2014) because of the high water content of the cyano-fertilizer. It is unlikely however, that this was the cause of the relatively higher fruit yield and lower vegetative growth, as Mitchell and Chambers (1982) found that an increase in water supply led to similar yields but increased vegetative growth so long as sufficient water was provided during critical fruit growth periods. The irrigation at Farm B was managed to avoid water-stressing the trees during the fruit developmental stages, meaning the yields should not have been limited by water availability. However, in our study, the higher-yielding treatments were those which received the extra water from the cyano-fertilizer, and higher vegetative growth was found in the plots without the additional irrigation water. Due to the discrepancy in results, it is likely that the differences were due to the cyano-fertilizer itself, rather than the extra water the plots received.

The exact mechanism which the cyano-fertilizer used to increase yields is not clear. In peaches, yields are governed by hand thinning practices, and vegetative growth and pruning in the previous year. These can all be ruled out on the basis of the RCBD which should account for these sources of variability, because there was nothing visibly different about the different treatments, and the crews doing the pruning and thinning had no prior knowledge of the experimental layout. There is a possibility that a nutrient deficiency caused more fruit to drop

mid-season in the No-Cyano treatment. Because of the relatively small amount of nutrients applied with the cyano-fertilizer (Table 4), the cyano-fertilizer may have allowed nutrients such as Fe to be taken up more easily by the trees. Siderophores are 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. In 2014, there were no significant differences in basal leaf Fe, Zn, or Mn concentrations (Table 9) found among treatments. In 2014, no attempt was made to compare chlorosis among treatments. Also, leaf tissue concentrations were tested only on basal leaves and not distal leaves, where a Fe deficiency would more likely be present. For these reasons it is impossible to be certain as to the cause of the fruit yield increase in Cyano-Manure plots. Although there is a possibility that phytohormones present in the cyano-fertilizer could have an effect on plants, it is unlikely that this influence yields at Farm B, because the fruit set and thinning practices determined the maximum fruit per tree.

In 2015 there were no significant differences in yield or TCSA for either the 2014 fertilization section or the 2015 fertilization section. This was influenced by the low fruit set, and heterogeneity of fruit set at both sites as a result of the April 3, 2015 freezing event during bloom. The freezing event likely had a similar effect on the results as did the aphid infestation on Farm A in 2014. A slight trend towards higher leaf nutrient concentrations in the High Manure treatments was seen in the 2015 fertilization section (Table 10). Because stored N is used for much of the vegetative growth, and yield effects are due to adequate numbers of fruiting sites for the following year, the N treatments are more likely to influence the following year's yields and growth (Taylor and van de Ende, 1969).

Leaf Tissue Nutrient Concentrations and SPAD

In 2014 there were no differences detected in leaf tissue nutrient concentrations among treatments at Farm A (Table 8), potentially due to the aphid pest problems associated with that experiment. In the Farm B 2014 fertilization section there were significantly higher S and Cu concentrations in basal leaves, and higher P concentration in distal leaves of Cyano-Manure treatments in 2015 (Table 9). The trend towards higher leaf nutrient concentrations in Cyano-Manure treatments may be associated with the addition of nutrients from the growing media, which were necessarily added along with the cyano-fertilizer; however, this trend was not documented across all experiments.

SPAD data was collected on Farm B when chlorosis became apparent across the orchard by a visual appraisal in 2015, and chlorosis seemed less frequent in Cyano-Manure treatment group plots across both sections at Farm B. The chlorosis was more common in the younger leaves, closer to the tip of shoots. The effect of nutrient concentrations, especially Fe (Abadia and Abadia, 1993; Belkhodja et al., 1998) on leaf chlorophyll content is well established. However, across several species, it has been found that Fe chlorosis can be present while leaf Fe concentrations are relatively high through what is called the "chlorosis paradox" (Römheld, 2000). The chlorosis paradox refers to the apparent high Fe concentrations despite lower total Fe content per leaf, due to the relatively smaller size of Fe deficient leaves (Römheld, 2000).

A difference in SPAD readings was documented in the Farm B 2015 fertilization section (Figure 8), but not in the 2014 fertilization section in 2015. Deficiencies in Zn, or Mn are unlikely because both were present in concentrations above literature suggested deficiency ranges, 15 and 20 ppm, respectively (Johnson and Uriu, 1989; Table 13), and because neither had significant correlations to SPAD readings. There are two likely explanations for the

differential chlorosis ratings evident in the data. First, Fe concentrations in leaf tissue are related to Fe chlorosis, since leaf Fe and SPAD were positively correlated (Table 11). Second, leaf S influenced chlorosis, since distal leaf S levels were significantly different between the No-Cyano treatment and the Cyano-Manure treatment group (Table 10).

It would be expected, if Fe deficiency alone were the cause of the chlorosis, that significant differences in SPAD rating between treatment groups would be accompanied by differences in distal leaf Fe concentration, but this was not the case (Tables 9 and 10). Also, according to Johnson and Uriu (1989), there appears to have been an adequate concentration of leaf Fe (above 60 ppm), although later than ideal timing of leaf sampling may have affected the result. The apparent adequate Fe nutrition seen in the Farm B 2015 fertilization section is most likely because of the chlorosis paradox, the chlorotic leaves being relatively smaller, inflating the Fe concentration, despite low per leaf Fe amounts (Römheld, 2000). This suggests that Fe chlorosis monitoring should be done on a per leaf basis as well as a concentration basis. The possibility that high nutrient concentrations can be present simply because the leaf size is smaller, should be taken into account for all nutrients, because if any nutrient is limiting and reduces the size of the leaf, all other nutrients will also be of disproportionately high concentration. The positioning of the chlorosis on the younger growth, indicates that the deficiency was of an immobile nutrient, and was most likely Fe. As mentioned earlier the positioning of the chlorosis on the younger growth may have been associated with siderophores in the cyano-fertilizer that would allow Fe to be more available to the trees.

Leaf tissue K concentration has been shown to increase with increasing levels of Fe chlorosis (Abadia et al., 1985; Belkhodja et al., 1998), which is consistent with our finding in the 2015 fertilization section (R=.662, P=.007). Abadia et al. (1985) found the P/Fe ratio was higher

in leaves with greater levels of chlorosis, which was supported by the correlation data in our study (Table 11). Abadia et al. (1985) claimed that the K/Ca Ratio is a better predictor of the Fe status of leaves; however, in our study the K/Ca ratio had a slightly weaker correlation with SPAD. This evidence would suggest that Fe is the cause of the chlorosis, despite the lack of significant distal leaf Fe concentration differences between treatment groups. Also, Fe being the cause of chlorosis would be supported by the finding that there was not a difference found in the second year of the 2014 fertilization experiment at Farm B, since Fe is an immobile nutrient and, therefore, would be lost at leaf fall.

In the Farm B 2015 fertilization section, the higher S concentrations in Cyano-Manure treatments may have been made higher because of the S present in the sulfates of Mg, Fe, Mn, Zn, and Cu, which are present in the nutrient media (Barminksi et al, 2016), however the amount of S added in the manure was higher than the amount added with the cyano-fertilizer (Table 4). Johnson and Uriu (1989) suggested that S is most commonly found in organic forms in soil which are converted to the inorganic form SO_4^{2-}. Since there is high (roughly 4.8%) organic matter in the top 15 cm of the soil at Farm B (Table A1 and A2), this suggests that S deficiency is unlikely.

For the fruit quality characteristics, there was a difference detected in fruit juice pH; however, the ratio of SSC:$[H^+]$ was not significantly different among treatments. For this study the assumption was made that the SSC:$[H^+]$ ratio can be considered to follow the same trends as the commonly cited SSC:TA ratio. It is understood that SSC, TA, and SSC: TA ratio can have an effect on consumer preference (Crisosto and Crisosto, 2003); however, consumer preference varies based on cultivar.

Farm C

The lack of compelling significant differences seen at Farm C may have been due to the two prior foliar fertilizations which had taken place on the farm prior to the initiation of the experiment. Unfortunately, the freezing event in Hotchkiss, CO left us with few options as sites for experimentation. However, the amount of fertilizer which was applied earlier in the season accounted for 50% of what was applied in a typical season **(**Table 7); therefore, it is reasonable to expect that there would still be enough remaining in the fertilizer budget to allow for an effect to be seen. Given this, it is reasonable to say that each fertilizer treatment performed equally well in terms of fruit yield and TCSA growth.

Some differences were seen among treatments in regards to leaf tissue nutrient concentration. The Fish Foliar treatment was higher in leaf Fe concentration than the Fish Drench treatment. Because of the prior fertilizations from earlier in the season, it is reasonable to assume that the leaves in question were sufficiently fertilized to avoid the previously discussed situation where leaf size was limited due to nutrient limitations, thereby distorting nutrient concentration data. Whereas it seems these data can be considered accurate, it is also important to note that the nutrient limits were still within the sufficiency range in almost every case according to Johnson (2008). In the case of Fe, the Fish Drench treatment was just below the sufficiency range; however, all other leaf nutrient concentrations were of sufficient concentration. This indicates that the fertilizers being tested were all adequate to provide the necessary nutrients in the season tested. One weakness of leaf tissue analysis in this case is that the leaf tissue analysis was done mid-season and before the large increase in cyanobacterial N fixation, meaning much of the cyano-fertilizer application occurred after nutrient analysis.

Although the circumstances of the experiment may have masked results, the results of this experiment indicate that there was little, if any, difference among the treatments in terms of viability as fertilizers for peach orchards.

Cyanobacterial Biofertilizer Production

Throughout both seasons at Farms A and B, the N fixation within the system was lower than expected. This could be the result of raising a culture that was adapted to another geographic region, antagonistic effects of other contaminating microorganisms within the raceway, or some other limiting factor such as excessive light. These factors may have limited the amount of N from the cyano-fertilizer available for application to 11.4 and 4.9 kg N/ha on Farm A and Farm B, respectively, as opposed to the target amount of 28 kg N/ha. Ultimately, this means only a small percentage of N available to the plants was from the cyano-fertilizer on Farms A and B. With the possibility that siderophores were released into solution to chelate Fe for the cyanobacteria, the cyano-fertilizer may have increased the availability of Fe to the trees.

On Farm C, the N production was higher at the end of the season with over 50% of the cyano-fertilizer N applied on this farm taking place in the final application (Table 1). This was possibly due to different growing conditions and the addition of 36% extra shading for the raceway which was added on July 24, 2015, and which coincided with the increase in N fixation. This suggests that more research should be done on the effect shading may have upon N fixation and biomass production within a cyano-fertilizer raceway. Possible reasons for increased production with a higher amount of shading may include avoiding excessive light which would require production of light protection compounds, or a decrease in the temperatures the cyanobacteria were exposed to in the raceway ponds.

Unfortunately, it is likely that the increase in N fixation happened at such a late point in the season that it would not affect the outcome of the experiment in a significant way.

CONCLUSION

Some of the literature suggests that N fertilization in peaches tends to have a higher influence over the following year's yields, rather than on the current year's growth, because of the use of stored N for much of the current year (Taylor and van den Ende, 1969). This is unfortunate, because the circumstances surrounding the experiments, the April 3, 2015 freezing event in Hotchkiss, CO, and the 2014 aphid infestation on Farm A, prevented a comparable reproduction of the experiment in the second year. As a result, treatment effects may have been masked by low nutrient demands due to low fruit yields. The early season fertilizations before experimentation at Farm C also may have masked results. For future research on N fertilizers in peaches, it is necessary to collect data for at least two consecutive seasons in order to capture the effects that fertilization has upon the following season.

At Farm B in 2014 Cyano-Manure treatments had higher yield and lower TCSA growth than the No-Cyano treatment. While it is unknown what the cause of the yield difference was, it may have been due to a prevention of fruit drop due to some micronutrient deficiency such as Fe. And it is likely that the lower TCSA growth followed the higher fruit count, because of the nutrient allocation being directed to the fruit to a greater degree. The increase in SPAD, at Farm B in the 2015 fertilization section, in Cyano-Manure treatments suggests that cyano-fertilizer may have increased Fe uptake by the trees, since SPAD was also positively correlated with distal leaf Fe concentration.

The increase in cyanobacterial N fixation with the additional shading should be investigated further to determine whether the effect may have been due to lower light levels, or temperature differences in the raceway pond.

TABLES

Table 1. Dates and N amounts of cyano-fertilizer applications made to Farms A, B, and C throughout 2014 and 2015.

	Cyano-Fertilizer Applications	
Farm/Section	Date of Application	Amount of N Applied
		---------kg/ha---------
Farm A	June 4 2014	2.11
	June 18 2014	1.49
	June 27 2014	1.38
	July 8 2014	1.99
	July 18 2014	2.26
	July 30 2014	2.21
Farm B 2014 Fertilization Section	May 29 2014	0.76
	June 20 2014	0.67
	June 25 2014	0.58
	July 4 2014	0.44
	July 7 2014	0.49
	July 19 2014	0.99
	July 232014	0.38
	July 31 2014	0.60
	August 1 2014	1.11
Farm B 2015 Fertilization Section	June 30 2015	0.92
	July 16 2015	2.14
	August 4 2015	1.88
Farm C[1]	June 19 2015	0.85
	June 20 20 2015	0.72
	July 9 2015	1.52
	July 24 2015	1.84
	August 12 2015	5.85

[1]N application amounts from cyano-fertilizer also correspond to the N amounts applied for treatments: Conventional Foliar, Fish Drench, and Fish Foliar; within a 24 hour period of the cyano-fertilizer applications.

Table 2. Description of fertilizer treatments used on Farm A in 2014. Chicken Meal was True Organic 12-3-0 fertilizer which is composed of: feather meal, blood meal and bone meal of chickens.

Treatment Name	Year	Fertilizers in Treatment
Chicken Meal+Cyano	2014	112 kg N/ha from chicken meal; 11.4 kg N/acre from cyano-fertilizer
Chicken Meal	2014	112 kg N/acre from chicken meal

Table 3. Description of fertilizer treatments used in the 2014 fertilization section of Farm B, in 2014 and 2015. The dried chicken manure used was Richlawn 5-3-2.

Treatment Name	Year	Fertilizers in Treatment
High Manure+Cyano	2014	112 kg N/ha from dried chicken manure; 4.9 kg N/ha from cyano-fertilizer
Low Manure+Cyano	2014	84 kg N/ha from dried chicken manure; 4.9 kg N/ha from cyano-fertilizer
High Manure	2014	112 kg N/ha from dried chicken manure
High Manure+Cyano	2015	112 kg N/ha from dried chicken manure
Low Manure+Cyano	2015	84 kg N/ha from dried chicken manure
High Manure	2015	112 kg N/ha from dried chicken manure

Table 4. Nutrient amounts applied from dried chicken manure and cyano-fertilizer on farm B in 2014. The amounts listed correspond to treatments which contained 112 kg N/ha from dried chicken manure, and 4.9 kg N/ha from cyano-fertilizer.

	Fertilizer Nutrients								
Fertilizer	N	P	K	Ca	S	Fe	Zn	Mn	Cu
	----------kg/ha----------								
Dried Chicken Manure	112[1]	67.2[1]	44.8[1]	89.6[1]	6.9[2]	4.0[3]	0.5[2]	0.6[2]	0.5[2]
Cyano-fertilizer	4.9	N/A*	4.3	N/A*	0.65	0.098	0.001	0.01	0.0004

*Amount unknown due to gradual release from bone-meal in raceway
[1]Adapted from The Richlawn Company, 2016.
[2]Estimated from Chastain et al., 2003.
[3]Estimated from McCall, 1980.

Table 5. Description of fertilization treatments used in the 2015 fertilization section of Farm B, in 2015. The dried chicken manure used was Richlawn 5-3-2.

Treatment Name	Year	Fertilizers in Treatment
High Manure+Cyano	2015	112 kg N/ha from dried chicken manure; 4.9 kg N/ha from cyano-fertilizer
Low Manure+Cyano	2015	84 kg N/ha from dried chicken manure; 4.9 kg N/ha from cyano-fertilizer
High Manure	2015	112 kg N/ha from dried chicken manure

Table 6. Description of fertilization treatments used on Farm C in 2015. Cyano treatment was applied first, and the other fertilizers were applied within a 24 hour period to match the same N amount which was applied from cyano-fertilizer.

Treatment Name	Year	Fertilizers in Treatment
Conventional Foliar	2015	10.8 kg N/ha from Pro-Sol 20-20-20, foliarly applied
Cyano	2015	10.8 kg N/ha from cyano-fertilizer, applied to soil
Fish Drench	2015	10.8 kg N/ha from Alaska 5-1-1 fish emulsion, applied to soil
Fish Foliar	2015	10.8 kg N/ha from Alaska 5-1-1 fish emulsion, foliarly applied

Table 7. Total nutrient amounts applied in two fertilization events in April, 2015 prior to experimentation on Farm C. The fertilizer was Pro-Sol 20-20-20, and was foliarly applied to trees.

	Fertilizer Nutrients							
Fertilizer	N	P	K	Fe	Zn	Mn	Cu	B
	----------kg/ha----------							
Pro-Sol 20-20-20[1]	4.5	4.5	4.5	0.244	0.122	0.122	0.122	0.049

[1]Pro-Sol, 2013.

Table 8. Mid-season basal leaf nutrient concentrations as affected by application of cyano-fertilizer at Farm A in 2014. Treatments included: 112 kg N/ha from True Organic 12-3-0 chicken based fertilizer plus 11.4 kg N/ha from cyano-fertilizer (Chicken Meal+Cyano), and another treatment which included 112 kg N/ha from True Organic 12-3-0 fertilizer alone (Chicken Meal). Means with a common letter are not significantly different from one another, as determined by ANOVA (P<0.1).

	Leaf Tissue Analysis							
Treatment	N[1]	P[2]	K[2]	Ca[2]	S[2]	Zn[2]	Fe[2]	Mn[2]
	----------%----------					----------mg/kg----------		
Chicken Meal+Cyano	3.49a	0.284a	2.95a	2.24a	0.172a	41.2a	120.2a	29.4a
Chicken Meal	3.51a	0.280a	2.89a	2.58a	0.176a	26.4a	79.4a	29.8a

[1]Extracted using NAPT method P 2.20 and analyzed using ICP-AES.
[2]Extracted using NAPT method P 4.30 and analyzed using ICP-AES.

Table 9. Mid-season basal and distal leaf nutrient concentrations as affected by application of cyano-fertilizer at Farm B in 2014 and 2015, in the 2014 fertilization section. Means with a common letter are not significantly different from one another, as determined by orthogonal contrasts. Treatment groups included: one group (Cyano-Manure) which included both treatments which included cyanobacteria, and another group which included the chicken manure only treatment (No Cyano).

Leaf Tissue Analysis									
Leaf Type	Treatment Group	N[1,3]	P[2,4]	K[2,4]	Ca[2,4]	S[2,4]	Zn[2,4]	Fe[2,4]	Mn[2,4]
		----------%----------					---------mg/kg----------		
		2014							
Basal leaves	Cyano-Manure	2.73a	0.227a	3.11a	2.95a	0.412a	30.3a	116.a	58.2a
	No Cyano	2.77a	0.210a	2.91a	2.82a	0.384a	29.2a	103.6a	55.4a
		2015							
Basal Leaves	Cyano-Manure	2.60a	0.252a	2.91a	2.63a	**0.171a***	30.2a	105.3a	31.9a
	No Cyano	2.62a	0.226a	2.77a	2.73a	**0.162b***	21.2a	73.8a	30.2a
Distal Leaves	Cyano-Manure	2.81a	**0.253a***	2.59a	2.75a	0.167a	22.4a	61.4a	36.5a
	No Cyano	2.74a	**0.223b***	2.44a	2.78a	0.162a	16.6a	55.4a	33.2a

*=significant to P<0.1
[1]Extracted using NAPT method P 2.20 and analyzed using ICP-AES.
[2]Extracted using NAPT method P 4.30 and analyzed using ICP-AES.
[3]N combusted and analyzed using LECO TruMac Nitrogen Combustion Analyzer.
[4]Extracted using nitric acid, hydrochloric acid, and hydrogen peroxide and analyzed using ICAP.

Table 10. Mid-season basal and distal leaf nutrient concentrations as affected by application of cyano-fertilizer at Farm B in the 2015 fertilization section. Means with a common letter are not significantly different from one another as determined by orthogonal contrasts. Treatment groups included: one group (Cyano-Manure) which included both treatments which included cyanobacteria, and another group which included the chicken manure only treatment (No Cyano).

		Leaf Tissue Analysis							
Leaf Type	Treatment Group	N[1]	P[2]	K[2]	S[2]	Ca[2]	Zn[2]	Fe[2]	Mn[2]
		----------%----------					----------mg/kg--------		
Basal Leaves	Cyano-Manure	2.60a	0.247a	3.00a	0.166a	**2.60b***	22.1a	75.1a	30.3a
	No Cyano	2.57a	0.251a	3.08a	0.162a	**2.74a***	32.2a	87.0a	32.4a
Distal Leaves	Cyano-Manure	2.74a	0.255a	2.56a	**0.166a****	2.79a	18.0a	65.6a	37.9a
	No Cyano	2.68a	0.249a	2.50a	**0.158b****	2.64a	19.2a	60.0a	38.2a

*=significant to P<0.1

**=significant to P<.05

[1]N combusted and analyzed using LECO TruMac Nitrogen Combustion Analyzer.

[2]Extracted using nitric acid, hydrochloric acid, and hydrogen peroxide and analyzed using ICAP.

Table 11. Correlation matrix showing correlative relationship of SPAD, SSC:[H^+] ratio, cyano-fertilizer N applied and distal leaf concentrations of K, Fe, S, Mn, Zn, P:Fe ratio and K:Ca ratio for the 2015 fertilization section of Farm B. Distal leaves were sampled for nutrient concentration on July 23, 2015 and SPAD was measured August 29, 2015.

	SPAD	Cyano-fertilizer N Applied	SSC: [H^+] Ratio	Distal leaf Fe Concentration	Distal Leaf S Concentration	Distal Leaf Zn Concentration	Distal Leaf Mn Concentration	Distal Leaf P:Fe ratio
Distal K:Ca Ratio	**0.78****	-0.31	-0.38	-0.36	**-0.47***	0.10	-0.39	**0.62****
Distal Leaf K Concentration	**-0.66****	-0.38	-0.37	**0.66****	-0.19	0.12	0.05	**0.84****
Distal P:Fe Ratio	**-0.59****	-0.13	-0.25	**-0.81****	-0.40	0.09	0.12	
Distal Leaf Mn Concentration	-0.36	-0.03	-0.15	-0.19	0.29	**0.45***		
Distal Leaf Zn Concentration	-0.44	-0.34	-0.30	0.21	0.11			
Distal Leaf S Concentration	0.37	**0.54****	0.40	0.35				
Distal leaf Fe Concentration	**0.62****	0.18	0.24					
SSC: [H^+] Ratio	**0.78****	0.37						

*=significant to P<0.1.

**=significant to P<.05.

Table 12. Mid-season basal leaf nutrient concentrations as affected by fertilizer treatment at Farm C in 2015. Treatments included: 10.8 kg N/ha from Pro-Sol 20-20-20 fertilizer foliarly applied (Conv. Foliar), 10.8 kg N/ha from cyano-fertilizer applied to soil (Cyano), 10.8 kg N/ha from Alaska 5-1-1 fish emulsion applied directly to the soil (Fish Soil Drench) and 10.8 kg N/ha of the fish emulsion applied as a foliar fertilizer (Fish Foliar). However, at the time of leaf sampling only 4.9 kg N/ha had been applied for all four treatments. Means without a common letter are different as determined by Duncan's Multiple Range Test ($P \leq 0.1$).

	Leaf Tissue Nutrient Analysis							
Treatment	N[1]	P[2]	K[2]	Ca[2]	S[2]	Zn[2]	Fe[2]	Mn[2]
	----------%----------					----------mg/kg----------		
Conv. Foliar	3.03a	0.211a	2.93a	2.41a	0.163a	**100.2ab**	**82.0ab**	44.2a
Cyano	3.06a	0.215a	2.88a	2.41a	0.163a	**96.0b**	**85.5ab**	47.3a
Fish Drench	3.00a	0.219a	2.89a	2.35a	0.165a	**107.7a**	**79.3b**	46.0a
Fish Foliar	3.08a	0.206a	2.97a	2.36a	0.166a	**94.6b**	**97.0a**	47.8a

[1]N combusted and analyzed using LECO TruMac Nitrogen Combustion Analyzer.
[2]Extracted using nitric acid, hydrochloric acid, and hydrogen peroxide and analyzed using ICAP.

Table 13. Deficiency and optimal levels of leaf nutrient concentrations.

	Leaf Nutrient Concentration	
Nutrient	Deficient Below[1]	Optimum Range[1]
	%	%
N	2.3	2.6-3.0
P	-	0.1-0.3
K	1.0	1.2
Ca	-	Over 1.0
Mg	0.25	Over 0.25
S	-	-
	mg/kg	mg/kg
Fe	60	Over 60
Mn	20	Over 20
Zn	15	Over 20
B	18	20-80
Cu	-	Over 4

[1]Adapted from Johnson and Uriu, 1989.

FIGURES

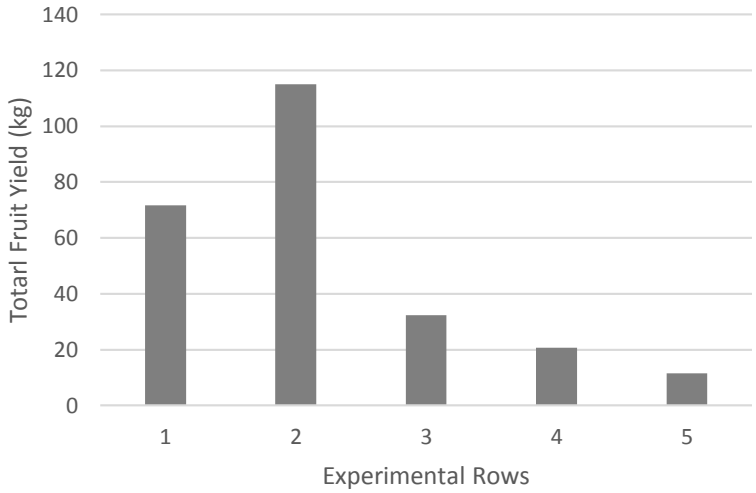

Figure 1. Total fruit yield per row, across both experimental treatments at Farm A in 2014. This figure shows the row to row variability in yield as a result of aphid damage. Each row included fruit picked from 3 trees from each of the two treatments.

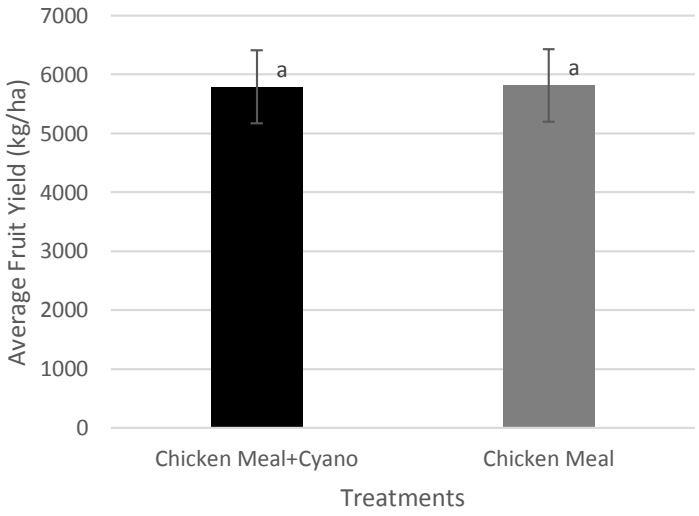

Figure 2. Comparison of the average fruit yield of treatments at Farm A in 2014. The treatments included: 112 kg N/ha from True Organic 12-3-0 chicken based fertilizer plus 11.4 kg N/ha from cyano-fertilizer (Chicken Meal+Cyano), and another treatment which included 112 kg N/ha from True Organic 12-3-0 fertilizer alone (Chicken Meal). Means without a common letter are different, as determined by ANOVA ($P \leq 0.05$), and error bars represent standard error.

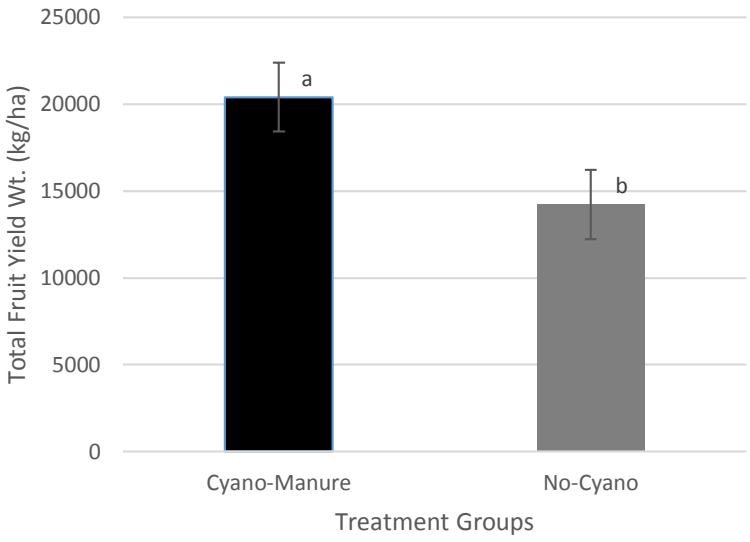

Figure 3. Comparison of the 2014 average yield of plots at Farm B, in the 2014 fertilization section, treated with Cyano-Manure and No-Cyano. Means without a common letter are different, as determined by ANOVA (*P≤0.05*), and error bars represent standard error.

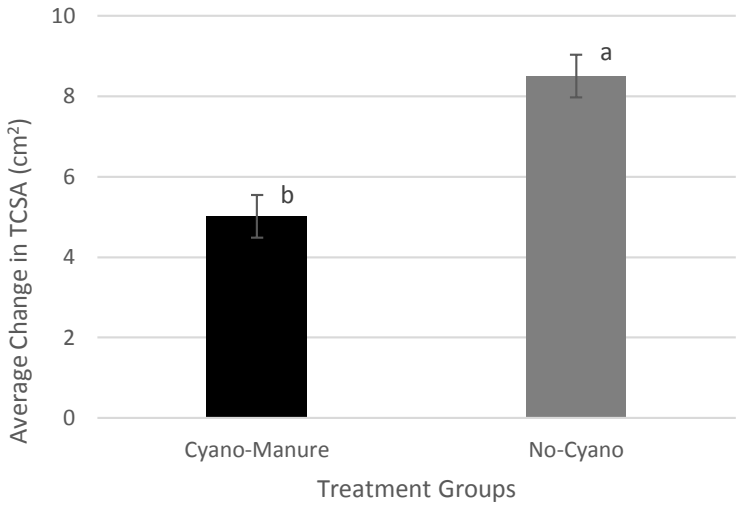

Figure 4. Comparison of the average growth in trunk cross sectional area of peach trees treated with Cyano-Manure and No-Cyano, on Farm B in 2014. Means without a common letter are different, as determined by ANOVA (*P≤0.05*), and error bars represent standard error.

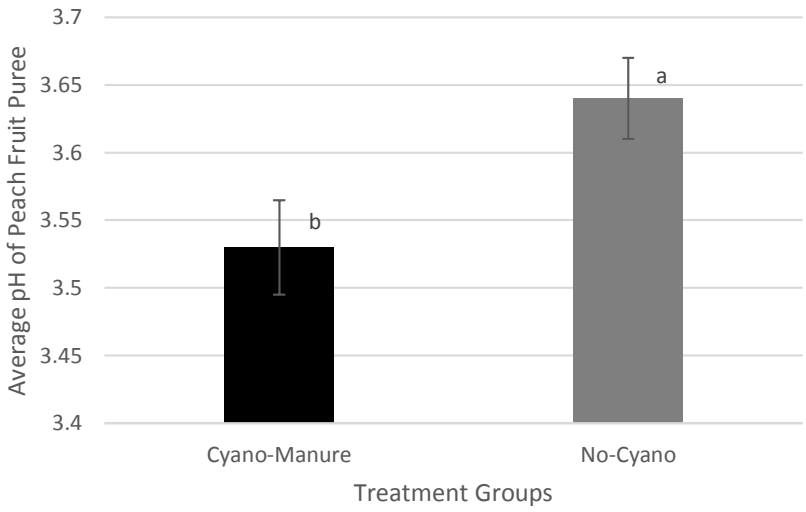

Figure 5. Comparison of the average pH of peach juice, in 2015, from plots treated Cyano-Manure and No-Cyano, on Farm B in the 2014 fertilization section. Means without a common letter are different, as determined by ANOVA (*P≤0.1*), and error bars represent standard error.

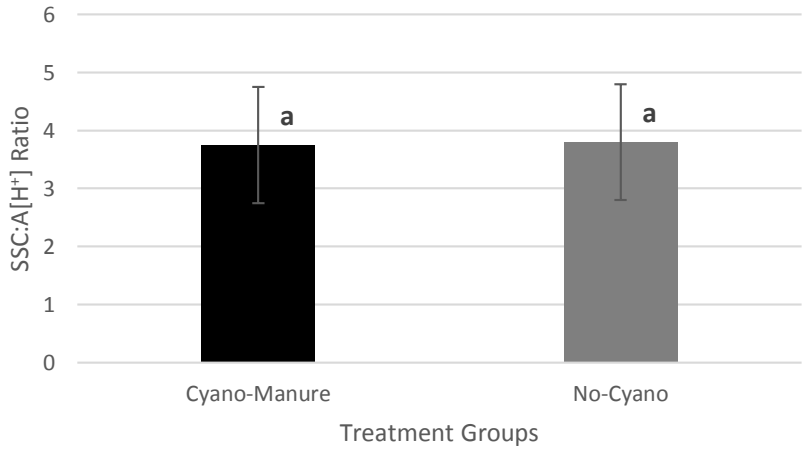

Figure 6. Comparison of the average soluble solids concentration:[H^+] ratio of peach fruit juice, in 2015, between plots treated with Cyano-Manure and No-Cyano, on Farm B in the 2014 fertilization section. Means without a common letter are different, as determined by ANOVA (*P≤0.05*), and error bars represent standard error.

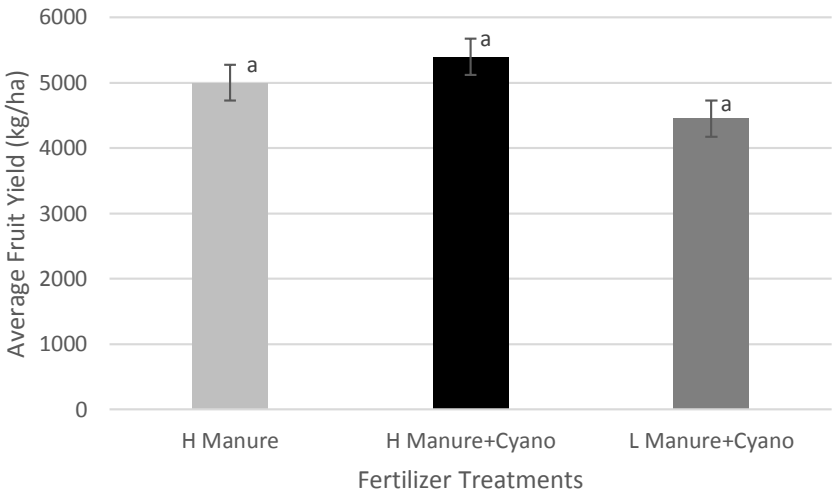

Figure 7. Comparison of the average fruit yields among treatments in the 2015 fertilization section of Farm B, for 2015. The treatments included: 112 kg N/ha from a dried chicken manure (H Manure), 112 kg N/ha from dried chicken manure plus 4.9 kg N/ha from the cyano-fertilizer (H Manure+Cyano), and 84 kg N/ha from dried chicken manure plus 4.9 kg N/ha from cyano-fertilizer (L Manure+Cyano). Means without a common letter are different, as determined by ANOVA ($P \leq 0.05$), and error bars represent standard error.

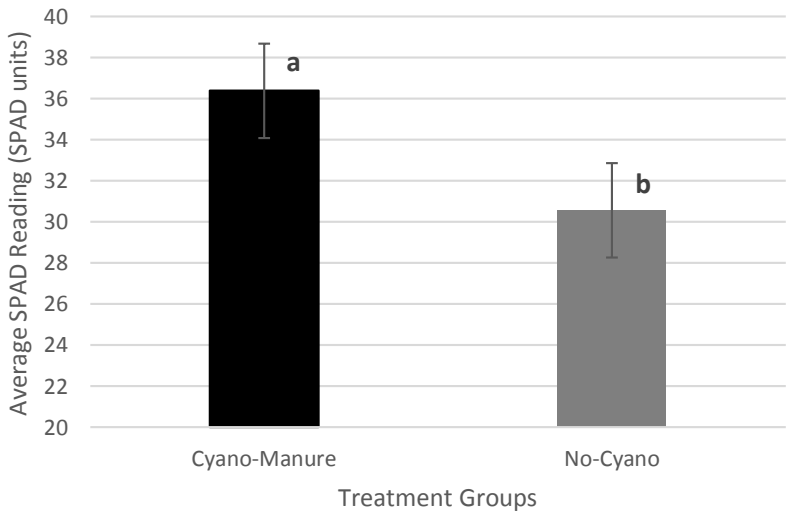

Figure 8. Comparison of the average chlorophyll rating in distal leaves between trees treated with Cyano-Manure and No-Cyano, as determined by SPAD reading, on Farm B in the 2015 fertilization section, on August 29, 2015. Means without a common letter are different, as determined by ANOVA ($P \leq 0.05$), and error bars represent standard error.

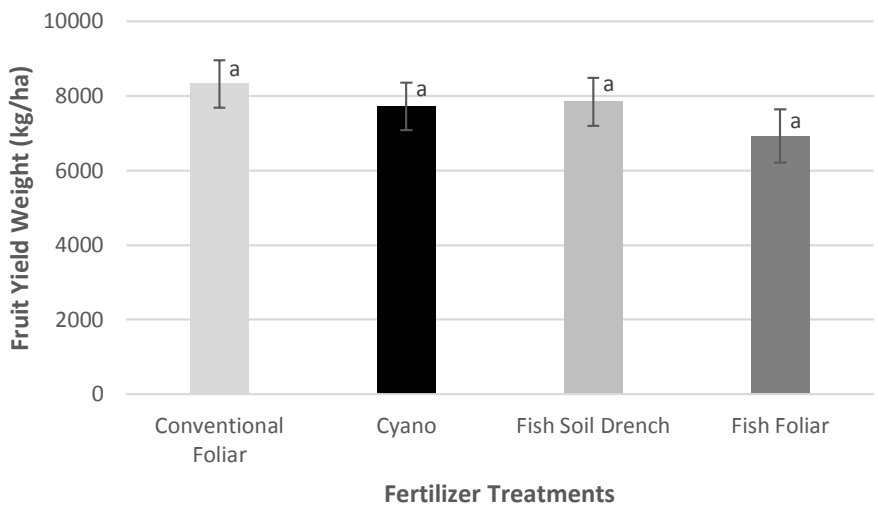

Figure 9. Comparison of fruit yield of treatments at Farm C in 2015. Treatments included: 10.8 kg N/ha from Pro-Sol 20-20-20 fertilizer foliarly applied (Conventional Foliar), 10.8 kg N/ha from cyano-fertilizer applied to soil (Cyano), 10.8 kg N/ha from Alaska 5-1-1 fish emulsion applied directly to the soil (Fish Soil Drench) and 10.8 kg N/ha of the fish emulsion applied as a foliar fertilizer (Fish Foliar). Means without a common letter are different as determined by Duncan's Multiple Range Test ($P \leq 0.1$), and error bars represent standard error.

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APPENDIX A

SOIL ANALYSES

TABLES

Table A1. Beginning and end of season soil analysis of Farm A. Values are means from ten separate soil samples taken April 26, 2014 and November 28, 2014, at a depth of 15 cm.

		Chemical Analysis									
Treatment	Year	pH[1]	OM[2]	NO_3^- N^3	P[4]	K[4]	S[4]	Ca[4]	Zn[4]	Fe[4]	Mn[4]
			%	----------mg/kg----------							
				Beginning of Season							
Chicken Meal+Cyano	2014	7.38	4.96	39.8	41.44	572.8	40.2	4605	9.43	12.1	12.7
Chicken Meal	2014	7.48	4.78	53.6	37.1	585	45.2	4618	7.20	9.1	11.5
				End of Season							
Chicken Meal+Cyano	2014	7.40	4.38	63.4	33.46	533.2	36.8	5019.2	4.818	12.0	9.16
Chicken Meal	2014	7.44	4.38	54	29.9	523	37.8	4775	4.92	10.5	9.62

[1]Determined by 1:1 soil water solution.
[2]Estimated by loss-on-ignition.
[3]Extracted using calcium phosphate and analyzed using Lachat FIA Analyzer.
[4]Extracted using DTPA and analyzed using ICAP.

Table A2. Beginning and end of season soil analyses of Farm B in the 2014 fertilization section, for years 2014, and 2015. Values are means of 15 separate soil samples taken April 26, 2014, and April 25, 2015, November 28, 2014 and October 24, 2015, at a depth of 15 cm.

	Chemical Analysis									
Treatment Group	pH[1]	OM[2]	NO_3^- N^3	P[4]	K[4]	S[4]	Ca[4]	Zn[4]	Fe[4]	Mn[4]
		%	----------mg/kg----------							
					2014					
					Beginning of Season					
Cyano-Manure	7.64	4.73	56.66	40.08	321	13.6	5668.2	3.54	6.18	11.23
No-Cyano	7.70	4.48	50.74	39.58	332	12.2	5522.2	2.90	4.76	9.7
					End of Season					
Cyano-Manure	7.55	4.65	45.84	56.67	411.5	13.8	6090.4	3.84	7.45	11.21
No-Cyano	7.54	4.88	45.76	60.4	441	13.6	5975.2	4.21	7.28	11.24
					2015					
					Beginning of Season					
Cyano-Manure	7.6	5.36	143.8	134.5	399.3	11.9	4506.5	3.81	4.48	4.12
No-Cyano	7.58	5.38	142.2	133.6	364.2	11.4	4210.2	3.65	3.76	3.32
					End of Season					
Cyano-Manure	7.8	7.64	80.8	81.23	439.1	12.2	3894	10.77	3.03	9.05
No-Cyano	7.76	7.58	60.2	66.4	439.2	11.4	4231	15.29	3.3	7.64

[1]Determined by 1:1 soil water solution.
[2]Estimated by loss-on-ignition.
[3]Extracted using calcium phosphate and analyzed using Lachat FIA Analyzer.
[4]Extracted using DTPA and analyzed using ICAP.

Table A3. Beginning and end of season soil analysis of the Farm B 2015 fertilization section, for 2015. Values are means of 15 separate soil samples taken in April 25, 2015, and at a depth of 15 cm.

	Chemical Analysis									
Treatment Group	pH[1]	OM[2]	NO_3^- N[3]	P[4]	K[4]	S[4]	Ca[4]	Zn[4]	Fe[4]	Mn[4]
		%	----------mg/kg----------							
Beginning of Season										
Cyano-Manure	7.7	5.0	140.7	102.6	340.5	11.3	4843	3.0	4.4	3.5
No-Cyano	7.7	4.5	148.0	110.5	332.5	11.8	5029	3.0	3.8	3.1
End of Season										
Cyano-Manure	7.7	7.0	70.4	63.9	423.6	10.9	4132.2	12.8	3.4	7.6
No-Cyano	7.8	6.8	76.8	72.4	390.3	10.3	4603.7	10.3	2.5	6.6

[1]Determined by 1:1 soil water solution.
[2]Estimated by loss-on-ignition.
[3]Extracted using calcium phosphate and analyzed using Lachat FIA Analyzer.
[4]Extracted using DTPA and analyzed using ICAP.

Table A4. Beginning and end of season soil analysis of Farm C. Values are means of 23 separate soil samples taken May 30, 2015 and October 24, 2015 at a depth of 15 cm.

	Chemical Analysis									
Treatment	pH[1]	OM[2]	NO_3^- N[3]	P[4]	K[4]	S[4]	Ca[4]	Zn[4]	Fe[4]	Mn[4]
		%	----------mg/kg----------							
Beginning of Season										
Conv. Foliar	8.1	1.6	54.2	59.3	426.8	44.2	3821.7	2.0	3.1	1.5
Cyano	8.1	1.5	49.3	55.2	518.0	40.7	3997.5	2.3	3.4	2.5
Fish Drench	8.1	1.5	48.2	58.5	455.8	109.5	3912.3	2.1	2.6	1.5
Fish Foliar	8.0	1.7	41.2	65.8	442.8	52.6	4094.2	2.2	2.7	1.6
End of Season										
Conv. Foliar	8.3	1.7	10.8	20.7	504.0	42.2	4230.5	7.8	3.6	2.9
Cyano	8.4	1.8	9.3	18.5	492.5	27.2	4115.8	8.2	2.8	3.2
Fish Drench	8.3	1.8	19.0	24.0	417.2	54.8	4235.3	9.2	4.0	2.6
Fish Foliar	8.4	1.9	8.8	20.6	451.8	35.2	4277.8	10.6	2.9	2.4

[1]Determined by 1:1 soil water solution.
[2]Estimated by loss-on-ignition.
[3]Extracted using calcium phosphate and analyzed using Lachat FIA Analyzer.
[4]Extracted using DTPA and analyzed using ICAP.

APPENDIX B

NITRATE MINERALIZATION

MATERIALS AND METHODS

Nitrogen mineralization was monitored in the cyano-fertilizer plots and the Fish Drench plots, on Farm C in 2015, using ion exchange resin membranes (Membranes International Inc., Ringwood NJ). Three pairs of cation and anion membranes (2.4 cm by 10 cm) were placed in the soil to a depth of 10 cm every two to three weeks immediately before each application, with the older membranes being removed at the time of subsequent installation. Nitrate and ammonium were extracted using 2.0 M KCl extraction (Michigan State University, 2009) and were analyzed by EcoCore Analytical Facilities at Colorado State University (Fort Collins, CO) using an Alpkem Flow Solution 4 (O.I. Analytical, College Station, Texas) using nitrate method 353.2 (U.S. Environmental Protection Agency, 1993) and DIN #38406 for ammonium determination.

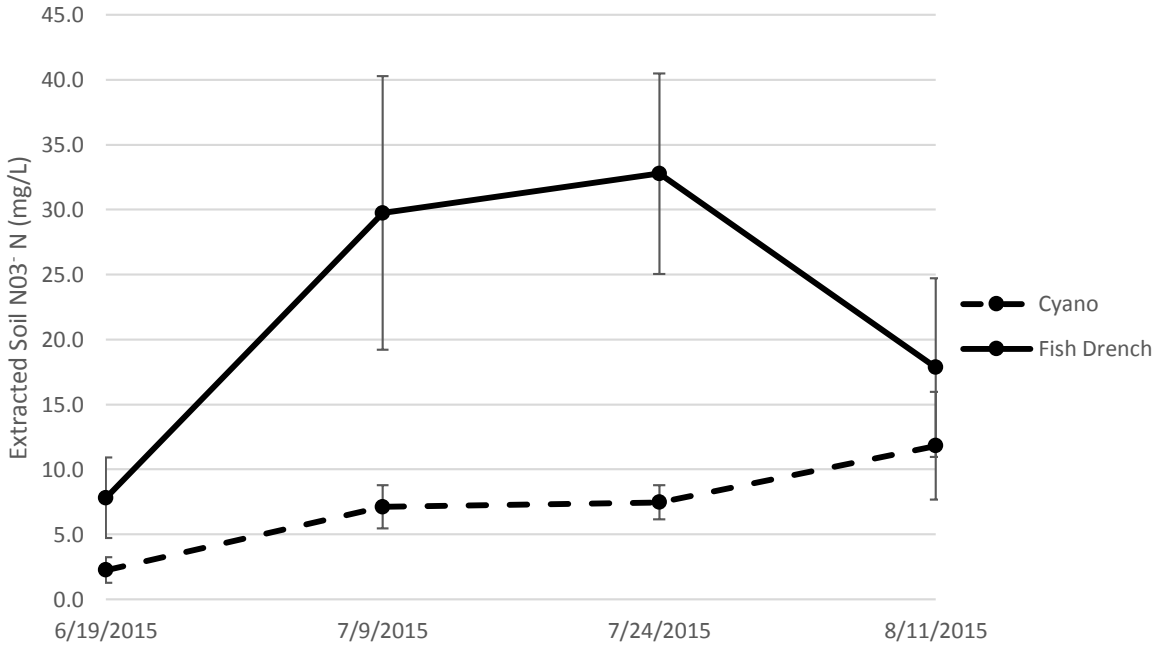

Figure B1. Amount of NO_3^- N extracted from anion exchange resins at various point throughout the experimental season.

DISCUSSION

There was only a negligible amount of NH_4^+ extracted from the cation exchange resins; and therefore, the data is not shown. A large discrepancy was seen between the Cyano and Fish Drench treatments for NO_3^- N extractions (Figure B1); hoswever, this is likely because of a weakness in the design of the experiment rather than being an accurate estimation of the mineralization within the soil. Because the application methods of Cyano and Fish Drench treatments varied, it is likely that more of the Fish Drench fertilizer came into contact with the resin strips. Fish Drench was applied in strips with a hand sprayer and relatively little water, while Cyano was applied via drip line with emitters spaced every 30 cm, and with a greater amount of water. In addition to the emitters not being directly above the resin strips, it is also possible that the extra water leached the NO_3^- below the 10 cm resin strip deeper into the soil. This mineralization methodolgy would be better suited for measuring differences between similar fertilization methods. This would minimize variation in the data by controlling critical variables.

APPENDIX C

CYANOBACTERIAL CULTURE COMPARSION

INTRODUCTION

Nine distinct cultures of cyanobacteria, isolated from soil-water samples in Western Colorado, were cultivated in a laboratory setting, until two were selected (WSR-3, WS2-D) as appearing to have more favorable characteristics for biofertilizer production. These characteristics included a tendency toward planktonic growth rather than aggregated growth, and N fixation rate. These cultures were then tested against the strain of cyanobacteria which is used in our cyanobacterial biofertilizer research (H-4). The purpose of this study was to determine whether one of the Western Colorado cultures could perform as well as H-4 in terms of growth rate and N fixation rate.

MATERIALS AND METHODS

On January 27, 2015 cultures were placed in 100 mL of Allen and Arnon Solution in 250 mL Erlenmeyer flasks. An adjusted volume of cyanobacteria was added to each flask so that the optical density of the culture was the same in each flask at the start of the experiment. The flasks were arranged in a 3x3 Latin Square Design (LSD), with 3 replicates per culture, on an orbital shaker under a grow light. Each culture's optical density (OD) at 550 nm and 595 nm was measured every 2-3 days after day five for two weeks, and N concentration was determined after 2 weeks.

RESULTS

H-4 had the highest OD throughout the entire experiment, while WS2-D and WSR-3 had similar OD (Fig. A1 and A2). The mean N levels were highest in H-4, and the variability was much less than what was found in WS2-D (Table A1). As a result, H-4 was considered to be the best option for cyanobacterial biofertilizer production.

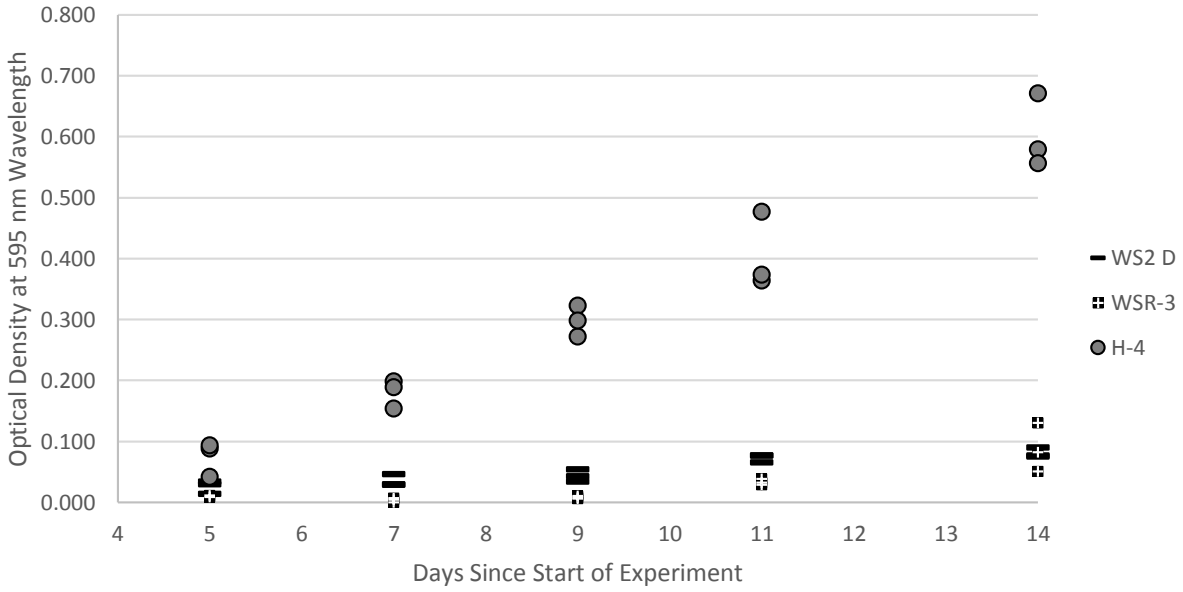

Figure C1. Comparison of the optical density (595 nm wavelength) of three distinct cultures of Cyanobacteria as it increased over a two week period. The cultures included the "control" culture which has been used since 2013 for cyanobacterial biofertilizer production (H-4), and two cultures which were isolated from water samples taken in Western Colorado (WS2-D, WSR-3). Cultures were grown in 250 mL Erlenmeyer flasks under a grow lamp.

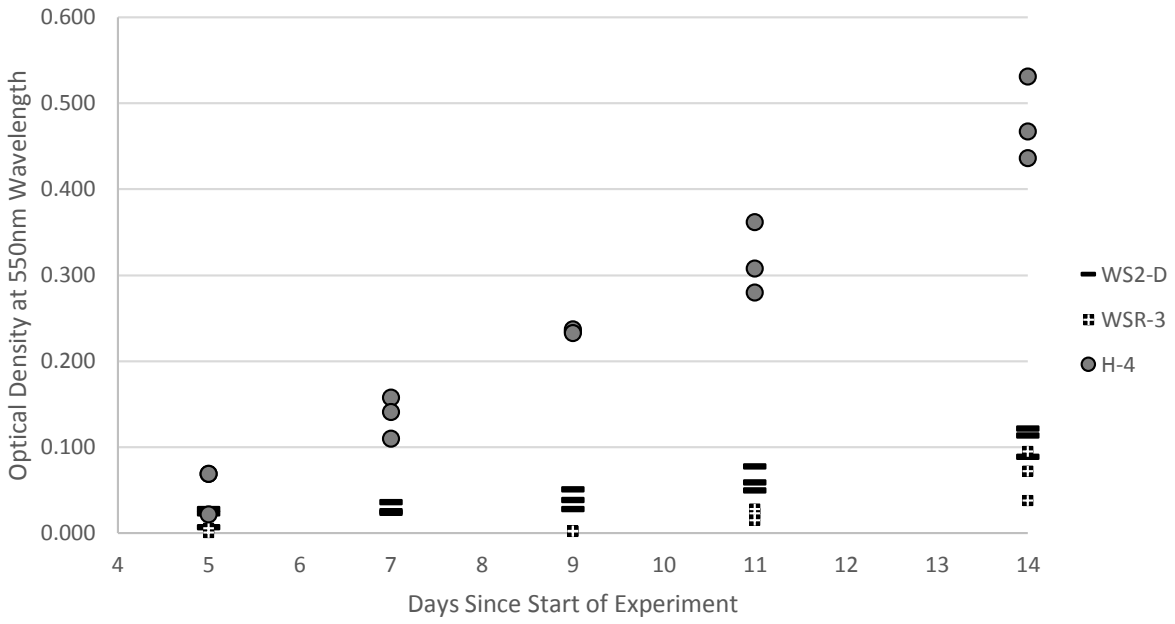

Figure C2. Comparison of the optical density (550 nm wavelength) of three distinct cultures of Cyanobacteria. The cultures included the "control" culture which has been used since 2013 for cyanobacterial biofertilizer production (H-4), and two cultures which were isolated from water samples taken in Western Colorado (WS2-D, WSR-3). Cultures were grown in 250 mL Erlenmeyer flasks under a grow lamp.

Table C1. Means and standard deviations of the N concentration of three separate strains of cyanobacteria, after 2 weeks of growth in a laboratory under a grow light.

Cyanobacterial Culture	N Concentration	Standard Deviation
	----------mg/kg----------	
H-4	23.6	3.6
WSR-3	6.7	2.2
WS2-D	15.9	17.7