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Cyanobacterial Biofertilizer as a Supplemental Fertilizer for Peaches: Yield, Trunk Growth, Leaf Nutrients and Chlorosis

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Abstract

Nitrogen is the nutrient which has the greatest effect on peach (*Prunus persica*) productivity and quality. Carbon assimilation is dependent upon adequate levels of N in leaf tissue. Cyanobacteria can fix gaseous N from the atmosphere enzymatically, and this N fixation can be exploited in a cyanobacterial biofertilizer production system, which allows farmers to grow their own N source with relatively small energy inputs. Cyanobacterial biofertilizer (*Anabaena* spp.) was grown on an organic peach farm in Hotchkiss, Colorado, and applied to a peach orchard as a supplement to dried chicken manure. Peach fruit yield, trunk cross sectional area, leaf tissue nutrient concentrations, and soil plant analysis (SPAD) chlorophyll meter values (an estimate of leaf chlorophyll concentration) were measured. Chicken manure fertilization supplemented with cyanobacteria had higher yield than manure alone. Trunk cross sectional area increased less for treatments with cyanobacteria. Contrasting treatments which received cyanobacteria with those that did not marginally significant increases in S, P, and Cu leaf tissue concentration were seen, and decreases in Ca concentration. SPAD values were positively correlated with leaf Fe concentration, indicating that chlorosis may have been due to Fe deficiency. Overall, insufficient evidence was found to suggest cyanobacterial biofertilizer would be beneficial use of resources for peach growers.

Cyanobacteria can be grown on-farm using organic nutrient media (Benemann, 1979; Barminski et al., 2016), and has been shown to be an effective organic fertilizer in annual vegetable crops (Asmamaw et al., 2019; Wenz et al., 2019; Yoder and Davis, 2020). *Anabaena* spp. and other types of cyanobacteria use nitrogenase to fix atmospheric N_2 gas, using specialized cells called heterocysts (Fay, 1992; Gallon, 1992; Thiel and Pratte, 2001). Prior research has shown that cyanobacterial biofertilizer (CF) can lead to higher yields than composted steer manure in lettuce (Sukor, 2013). CF decreased soil pH and increased Fe and Zn concentrations in kale, hot pepper, and maize (Asmamaw et al., 2019). On-farm production of N fertilizer can decrease the energy involved in transporting fertilizers from off-farm locations and can be a source of sustainable N fertilizer for organic cropping systems. Cyanobacteria are commonly found throughout the world, and their adaptation across environments suggests locally isolated cultures could be successfully grown in diverse locations.

Nitrogen is the most commonly limiting nutrient in plants and the nutrient applied in the greatest amounts in the majority of crops. Nitrogen fertilization of peach trees results in higher yield, larger fruit, and delayed maturation (Rader et al., 1985; Saenz et al., 1997; Rufat et al., 2010). Additionally, Cain and Meilenbacher (1956) found increases in yield 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

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was attributed to an increase in vegetative growth, creating more potential fruiting sites in the year after fertilization. Soil plant analysis (SPAD) chlorophyll meter values (an estimate of leaf chlorophyll concentration) increased in mature peach trees with increasing N fertilization (Olmstead et al., 2015). Carbon assimilation is highly correlated with N concentration 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 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 leaf N concentration and shoot growth were 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 optimum N fertilization timing of stone fruit trees should be spring or autumn or a combination of the two (Taylor and May, 1967; Bi et al., 2003; Jordan et al., 2013). Researchers have shown that trees, especially mature ones, have the ability to buffer their N supply with internal storage, rather than being completely dependent on 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 was mobilized and stored in woody tissue during leaf senescence (Niederholzer et al., 2001).

Along with N, other nutrients such as Fe are important for peach production. Iron chlorosis results from low amounts of chlorophyll within a leaf due to Fe deficiency (Abadia and Abadia, 1993). In Western Colorado, alkaline soils and irrigation water contribute to the prevalence of iron chlorosis in peach. Moderate Fe chlorosis was related to decreased peach fruit yield by up to 83% (Alvarez-Fernandez et al., 2011). SPAD meters were used to estimate leaf chlorophyll content per leaf area and to quantify the extent of chlorosis on peach leaves (Belkhodja et al., 1998; Alvarez-Fernandez et al., 2011). Cyanobacteria are known to release siderophores in Fe-limiting environments, and siderophores chelate Fe into bio-available forms (Wilhelm and Trick, 1994).

The objective of this research was to evaluate the effectiveness of CF as a supplemental N fertilizer source in conjunction with dried chicken manure within a commercial grower's management system. The apparent effect of the CF upon chlorosis was also investigated to determine whether the effect was caused by increased Fe concentration in leaf tissue or other possible factors. We hypothesized that CF application would lead to equal or greater yield and vegetative growth in peach in comparison to no CF application. We also hypothesized that CF application would result in peach leaf tissue nutrient concentrations, specifically N and Fe, which would be 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

Cyanobacterial biofertilizer production and application. In 2014 and 2015, cyanobacteria (*Anabaena* spp.) were grown at an organic fruit orchard in Hotchkiss, CO. The cyanobacteria were grown in paddlewheel-agitated, 25-cm deep raceway ponds of 2,366 L to 4,921 L in volume, using an organic nutrient media (Barminski et al., 2016). Nutrients were added to the raceway individually for NaCl, KCl, and $MgSO_4 \cdot 7H_2O$, while $FeSO_4 \cdot 7H_2O$ was first mixed with distilled water before adding, and the other micronutrients [$MnSO_4 \cdot H_2O$, $Na_2Mo4 \cdot 2H_2O$ (99% purity), $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, H_3BO_3 and $CoCO_3$] were added simultaneously. One

nutrient was added to the raceway every 2-5 minutes with the paddlewheel circulating the water the entire time. The cyanobacteria were applied directly to the field as a fertilizer after a growth period of 12 to 18 days, when the cyanobacteria culture was healthy. Health of cyanobacteria population was evaluated through microscopic culture contamination appraisals, as well as both an optical density measurement of the culture at 550 nm and a total Kjeldahl nitrogen (TKN) measurement using a Hach DR 3900 spectrophotometer (Hach Co., Loveland, CO). Five applications were made to the orchard per season when the culture was deemed to be mature based on optical density.

The application of the biofertilizer was made using a sump pump connected to an irrigation system which mimicked the micro-sprinkler irrigation systems used on the farms. In 2014, five applications of CF were made from 28 May- 1 Aug., and in 2015 five applications were made from 19 Jun.-12 Aug. The biofertilizer was pumped to a 946 L tank which was taken to the application site directly after being filled from the raceway pond. Volume applied was recorded and multiplied by TKN concentration to determine the amount of N applied. Throughout an application, roughly 90 percent of a raceway pond volume was applied. Tap water and a proportionate amount of nutrient media were then added to the pond to start a new CF growing period.

Plot layout and treatments. 'Suncrest' peaches grafted on a 'Lovell' rootstock and planted in 1999 were managed organically throughout the life of the orchard. The spacing was 1.6m x 4.6m between trees and rows, respectively. The soil had a pH of 7.6, and the soil type was a Mesa loam which is classified as mesic Typic Calciargids (Natural Resources Conservation Service, 2008).

Experimental units 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. Three treatments were applied: 1) "High Manure" (NC) was 112 kg N/ha from Richlawn 5-3-2 (Platteville, CO) a dried poultry manure, 2) "High Manure+Cyano" (HMC) was 112 kg N/ha from Richlawn 5-3-2 with 4.9 kg N/ha from CF, and 3) "Low Manure+Cyano" (LMC) was 84 kg N/ha from Richlawn 5-3-2 with 4.9 kg N/ha from CF. In addition to N, other nutrients were applied with the CF which originated with the growing medium used (Table 1). In 2015, a second experiment was added (2015 fertilization section), comprised of the same treatment applications, in separate reps within the same orchard. In 2015 the reps from the 2014 fertilization section treatments continued to be evalu-

Table 1. Nutrient amounts applied from dried chicken manure and cyanobacterial biofertilizer (CF) 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 cyanobacterial biofertilizer.

	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]
CF	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).

ated for residual effects, but they were only given their respective amounts of Richlawn 5-3-2, excluding the CF. On 3 April of 2015 a freezing event killed most of the blossoms on the trees in the orchard, resulting in 70% yield and fruit count reductions compared to the 2014 season, since temperatures dropped to approximately -3.8 °C for at least 3 hours (Colorado State University, 2015).

Measurements. Tree trunk cross sectional area (TCSA) was estimated from trunk circumference measured 20 cm above the orchard floor in the spring prior to fertilizer application, and in the autumn after the growing season had ended and the difference between the two measurements was calculated.

Sampling for midseason leaf tissue nutrient analysis followed the protocol given by A & L Western Labs (www.al-labs-west.com). The sampling dates were in mid-July both years. 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-section of first year shoots, with petioles attached, and were not washed before sending to the 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, Zn, Fe, Mn, and Cu (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 P, S, Na, Zn, Fe, Mn, Cu and B concentrations 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). Mid-August 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.

Chlorosis was monitored in late Aug. 2015 because moderate to severe chlorosis was evident. 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. Each tree was divided into 4 quadrants based on compass directions. Five leaves were each scanned once per quadrant, and the mean of those measurements was recorded for each quadrant of each tree. Peaches were harvested into boxes, and total fruit count and fruit weight were recorded for each plot.

Statistical analysis. The data were analyzed as a Randomized Complete Block Design (RCBD) using SAS 9.4 (SAS Institute Inc., Gary, NC). Values tested were mean values of the three measurement trees within each plot. 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 CF (HMC and LMC) and comparing them to the treatment without CF (NC). Correlations between yield, SPAD, TCSA change, and leaf tissue nutrient concentrations were analyzed using the Corr procedure. ANCOVA was performed using JMP Pro 15 with treatment as the independent variable, and initial TCSA as a covariate for determining significance of differences in seasonal TCSA increase, and fruit count. Due to high variability in data caused by differences in peach fruit thinning, branch pruning and tree health, an alpha value of 0.10 was used as a critical significance level, and P-values below 0.10 but above 0.05 will be referred to as "marginally significant." Figures were created using Graphpad Prism 8.

Results

2014 fertilization section. In 2014, fruit yield was significantly higher in plots treated

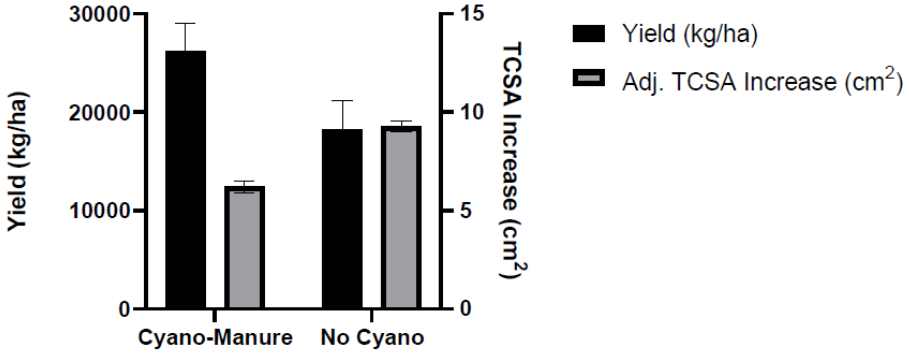

Figure 1. Comparison of the 2014 average yield and adjusted average change in trunk cross-sectional area (TCSA) of plots in the 2014 fertilization section, treated with cyanobacterial biofertilizer plus manure (CM) and manure alone (NC). TCSA increase was adjusted for initial TCSA and interaction with treatment. Error bars represent standard error as determined by ANOVA for yield and ANCOVA for adjusted TCSA increase ($P \leq 0.05$).

with both CF and manure (CM) compared to manure alone (NC) (P=0.0346; Figure 1). There was only a marginal difference found among individual treatment means using ANOVA (P=0.0937), with HMC, LMC, and NC having respective means of 26,194; 26,185; and 18,260 kg/ha. The same was true for average fruit count, which was significantly higher (P=0.0216) in CM compared to NC groups, and marginally different (P=0.0781; data not shown) between individual treatments. HMC, LMC and NC had respective mean fruit counts of 89.4, 87.5, and 62.0 fruit per tree. When adjusting for initial TCSA fruit count means were 78.6, 86.4 and 61.0 and insignificant (P=0.1642). In 2015, fruit yield and average fruit count were not affected by treatment (data not shown). No difference was seen in crop density (number of fruits per cm^2 TCSA) in 2014 or 2015 with respective means of 0.5 and 0.2 fruit per cm^2. No difference was seen in yield efficiency (yield per cm^2 TCSA) in 2014 or 2015 either with respective means of 0.12 kg/cm^2 and 0.08 kg/cm^2.

In 2014, TCSA increase was higher in NC than CM (Fig. 1), and higher than both individual treatments receiving CF supplementation, HMC and LMC (P= 0.0042 and 0.0004, respectively; data not shown) when adjusting for initial TCSA and interaction between initial TCSA and treatment. HMC, LMC and NC had respective mean increases in TCSA of 5.6, 4.3, and 8.5 cm^2 but adjusted means of 7.0, 5.1 and 9.3 cm^2 in 2014. In 2014 initial TCSA had a significant positive relationship with TCSA increase ($P \leq .0001$)). The interaction between initial TCSA and treatment were significant between HMC and NC only (P=0.0069) with a HMC having a slope 2.2% greater than the overall average, and NC having a slope 8.8% less than the overall average. Fruit count did not have a significant effect on TCSA increase. There were no significant differences indicating residual effects of the 2014 CF application on TCSA growth in 2015.

In 2014, basal leaf tissue nutrient concentrations were not affected by treatment; however, means tended to be higher in CM treatments than in NC treatment (Table 2). In 2015, marginally significantly higher basal leaf tissue S (P=0.0882) and Cu (P=0.0868; data not shown) concentrations were found in CM treatments than in the NC treatment. No differences were seen among individual treatments HMC, LMC, and NC with respective means of 0.174%, 0.168% and 0.162% for basal S and 19.2, 18.64 and 16.88 ppm for basal Cu. There were marginally signifi-

Table 2. Mid-season basal and distal leaf nutrient concentrations as affected by application of cyanobacterial biofertilizer (CF) 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 CM which included both treatments which included cyanobacterial biofertilizer, and another group which included the chicken manure only treatment (NC). Separate comparisons across treatment group were made for each leaf position and year.

		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	CM	2.73a	0.227a	3.11a	2.95a	0.412a	30.3a	116.a	58.2a
	NC	2.77a	0.210a	2.91a	2.82a	0.384a	29.2a	103.6a	55.4a
		2015							
Basal Leaves	CM	2.60a	0.252a	2.91a	2.63a	**0.171a***	30.2a	105.3a	31.9a
	NC	2.62a	0.226a	2.77a	2.73a	**0.162b***	21.2a	73.8a	30.2a
Distal Leaves	CM	2.81a	**0.253a***	2.59a	2.75a	0.167a	22.4a	61.4a	36.5a
	NC	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.

cantly higher distal leaf tissue P (P=0.0945) in CM treatments than in the NC treatment (Table 2), but not among individual treatments HMC, LMC and NC with respective means of 0.239%, 0.267% and 0.223%. Additionally, both basal and distal leaf tissue nutrient concentrations tended to be higher in CM treatments in 2015 compared to the NC treatment (Table 2).

In 2015, SPAD values did not differ for treatments, and SPAD was significantly and positively correlated to distal leaf P concentrations (r=0.643; P=0.0097; data not shown).

2015 fertilization section. In contrast to the first-year results in the 2014 fertilization section, there were no significant differences in yield or TCSA increase among treatments or between treatment groups in the 2015 fertilization section. There was a discrepancy in the mean yield per tree when comparing all treatments in 2014 to 2015 as mean tree fruit set was 80 fruit per tree in 2014 while only 25 in 2015 because of a lethal April frost event in 2015. Marginally significant differences were found in basal leaf tissue Ca (P=0.0600; Table 3) and B (P=0.0854; data not shown), with concentrations being higher in the NC treatment than in CM treatments, but this difference was not evident among individual treatments. There was no difference in means among HMC, LMC, and NC in basal leaf Ca with 2.57%, 2.63%, and 2.74% respectively or basal leaf B with 38.4, 38.9, and 44.4 ppm, respectively. There were no other significant differences among treatments or between treatment groups for basal leaf nutrient concentrations. Distal leaf S concentration was significantly higher (P=0.0388) in CM treatments compared to NC treatments, but not among treatments HMC, LMC, and NC with means of 0.168%, 0.164% and 0.158% respectively. There were no other differences among treatments for other nutrients (Table 3). No differences were seen in crop density (mean=0.13 fruit/cm^2), or yield efficiency (mean=0.08 kg/cm^2).

Table 3. Mid-season basal and distal leaf nutrient concentrations as affected by application of cyanobacterial biofertilizer (CF) 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 (CM) which included both treatments which included cyanobacterial biofertilizer, and another group which included the chicken manure only treatment (NC). Means were compared between treatment groups in separate analyses for basal and distal leaves.

		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	CM	2.60a	0.247a	3.00a	0.166a	**2.60b***	22.1a	75.1a	30.3a
	NC	2.57a	0.251a	3.08a	0.162a	**2.74a***	32.2a	87.0a	32.4a
Distal Leaves	CM	2.74a	0.255a	2.56a	**0.166a****	2.79a	18.0a	65.6a	37.9a
	NC	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.

SPAD values were significantly higher (P=0.0350) in CM treatments compared to the NC treatment (Figure 2), but not different among individual treatments with means for HMC, LMC and NC of 37.1, 35.7, and 30.6 respectively. SPAD was significantly and positively correlated to the CF N amount applied (r=0.57; P=0.0261) and distal leaf Fe concentration (r=0.62, P=0.0136; Table 4). Distal leaf K concentrations were negatively correlated to SPAD readings (r=-0.66, P=0.0072; Table 4). Significant correlations and marginally significant values were found between SPAD and P/Fe ratio (r=-0.59, P=0.0196; Table 4) and K/Ca ratio in distal leaves, respectively (r=-0.78, P=0.062; Table 4).

Discussion

Yield and growth. In 2014, while the CM treatments yielded significantly higher in terms of total fruit weight (Figure 1) and fruit count (data not shown) than the NC treatment, the NC treatment had greater TCSA growth, indicating that lower crop load may have contributed to greater vegetative growth (Fig. 1). ANCOVA revealed that the difference in vegetative growth was significantly related to the initial TCSA of the trees, since larger trees will generally have more growth,

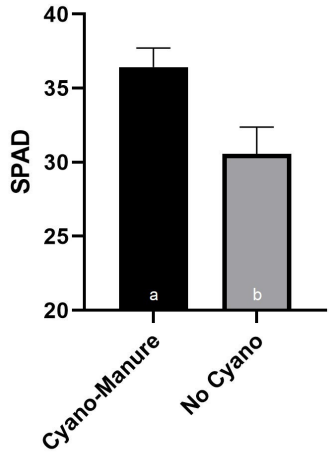

Figure 2. Comparison of the average chlorophyll rating in distal leaves between trees treated with cyanobacterial biofertilizer plus manure (CM) and manure alone (NC), as determined by SPAD reading, in the 2015 fertilization section, on August 29 of 2015. Means without a common letter are different, as determined by ANOVA (*P≤0.05*), and error bars represent standard error.

even though significant difference in initial TCSA between treatments was not seen with ANOVA (P=0.61). Also significant was the interaction between treatment and initial TCSA (P=0.0139), which revealed that for NC TCSA increased less for larger trees while in HMC and LMC larger trees tended to grow more relative to smaller trees within the given treatment. This difference is surprising however, when considering how the large NC trees still grew more than the large HMC and LMC trees, it is less likely not relevant for the purposes of this study.

Given that fruit counts adjusting for initial TCSA were not different, this indicates that differences in yield were because of differences in initial tree size. It should be noted that total fruit set in 2014 averaged 80 fruit per tree, while in 2015 across both sections and all treatments mean fruit set was only 25 fruit per tree because of a spring frost event. Given the damaging frost event, differences in fruit set were undermined because the main factor determining fruit set was the frost damage.

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 freezing event during bloom in 2015. A slight trend towards higher leaf nutrient concentrations in the CM treatments occurred in the 2015 fertilization section (Table 3).

Leaf tissue nutrient concentrations and SPAD measurements. In the 2014 fertilization section, there were significantly higher S and Cu concentrations in basal leaves and higher P concentration in distal leaves from CM treatments in 2015 (Table 2). The trend towards higher leaf nutrient concentrations in CM treatments may be associated with the addition of nutrients from the growing media, which were necessarily added along with the CF, although the micronutrients were added in such small quantities that this may not be the case (Table 1).

SPAD data were collected when chlorosis became apparent across the orchard by visual appraisal in 2015, and chlorosis seemed less frequent in CM plots across both fertilization sections. Chlorosis was common throughout this orchard, likely because the soil pH of 7.6 was relatively high which makes nutrients such as Fe, Zn, and Mn less available to plants. Chlorosis was more common in the younger leaves, closer to the shoot tips. The effect of nutrient concentrations, especially Fe (Abadia and Abadia, 1993; Belkhodja et al., 1998), on leaf chlorophyll content is well established.

A difference in SPAD readings was documented in the 2015 fertilization section (Figure 2), but not in the 2014 fertilization section in 2015. Deficiencies in Zn or Mn are unlikely, because both were present in leaf concentrations above literature suggested deficiency ranges, 15 and 20 $mg \cdot kg^{-1}$, respectively (Johnson and Uriu, 1989), and because neither were significantly correlated to SPAD values. It should be noted that the correlations are likely lower than would be expected, because the SAS Proc Corr procedure does not partition block variation out of the residual error term. There are two likely explanations for the SPAD data. First, Fe concentrations in leaf tissue were related to chlorosis since leaf Fe and SPAD were positively correlated (Table 4). Second, leaf S influenced chlorosis, since distal leaf S levels were significantly lower in the NC treatment than the CM treatment group (Table 3).

In the 2015 fertilization section, the leaf S concentrations in CM treatments may have been 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 more than 10-fold higher than the amount added with the CF (Table 1). Johnson and Uriu (1989) stated 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 these heavily amended soils,

Table 4. Correlation matrix showing correlation coefficients (r) of SPAD, cyanobacterial biofertilizer (CF) N applied, and distal leaf concentrations of Fe, Mn, Zn, S, K, P:Fe ratio and K:Ca ratio for the 2015 fertilization section. Distal leaves were sampled for nutrient concentration on July 23 of 2015, and SPAD was measured August 29 of 2015.

	SPAD	Amount CF N Applied	Distal leaf Fe Concentration	Distal Leaf S Concentration	Distal Leaf Zn Concentration	Distal Leaf Mn Concentration	Distal Leaf P:Fe ratio
Distal Leaf Fe Concentration	**0.62****	0.18	~	0.35	0.21	-0.19	**-0.81****
Distal Leaf Mn Concentration	-0.36	-0.03	-0.19	-0.29	**0.45***	~	0.12
Distal Leaf Zn Concentration	-0.44	-0.34	0.21	0.11	~	**0.45***	0.09
Distal Leaf S Concentration	0.37	**0.54****	0.35	~	0.11	0.29	-0.40
Distal Leaf K Concentration	**-0.66****	-0.38	**0.66****	-0.19	0.12	0.05	**0.84****
Distal P:Fe Ratio	**-0.59****	-0.13	**-0.81****	-0.40	0.09	0.12	~
Distal K:Ca Ratio	**-0.78***	-0.13	-0.36	**-0.47***	0.10	-0.39	**0.62****

*=significant to P<0.1.
**=significant to P<.05.

it is possible that the CF increased microbial activity leading to an increase in S mineralization, rather than because of the S added to the rhizosphere. While the possible effects are speculative, microscopy confirmed a wide variety of microbial diversity in the CF besides cyanobacteria which included protozoa, algae, and bacteria. As such it is possible that this influx of various microbes may have affected nutrient uptake.

Conclusion

Because much of the N which is used in a given year comes from stored N within the tree, it may take multiple years to see the accumulated effects of N fertilization be expressed in peach (Taylor and van den Ende, 1969). Therefore, there may be greater differences after more successive years of CF application and observation. In 2015, results could have been masked due to variability and highly limited fruit set, due to frost damage which occurred at bloom time.

In 2014, CM treatments had higher yield and lower TCSA increase than the NC treatment. It is likely that the lower TCSA increase was a direct outcome of the higher fruit count. The increase in SPAD in CM treatments in the 2015 fertilization section suggests that CF may have increased Fe uptake by the trees, since SPAD was positively correlated with distal leaf Fe concentration. No detrimental effects were observed as a result of CF or the diverse population of microbes included therein, however there is little evidence that there is a beneficial impact which would outweigh the cost to peach growers. This study does prove a concept that the cyanobacterial biofertilizer can be produced and implemented on a commercial peach orchard.

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