

THE EFFECT OF CYANOBACTERIA BIOFERTILIZER ON WESTERN COLORADO ORGANIC PEACH QUALITY AND YIELD CHARACTERISTICS

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ABSTRACT

Nitrogen (N) is the nutrient needed by crops in the highest amounts and the production of synthetic N fertilizers contributes the highest proportion of carbon dioxide to the atmosphere, when compared to other sectors of agriculture. Cyanobacteria are naturally occurring in most ecosystems and fix nitrogen gas from the atmosphere into forms which are useable by plants. Cyanobacteria was applied along with irrigation water to organically farmed peach trees (*Prunus persica* cv. Suncrest) as a nitrogen fertilizer at two different peach orchards in Hotchkiss, CO. The cyanobacteria fertilizer was grown on farm, in 625 and 800 gallon pools enclosed within plastic hoop houses, and grown in batches for two weeks before applications. For unknown reasons the cyanobacteria fixed a lower amount of N than expected. The cyanobacteria were applied via a parallel micro-sprinkler system which mimicked the growers' systems in terms of spacing, emitter, and orifice size. The control treatment was the growers' standard practice application of 100 lb. of N per acre from the fertilizers True Organic, and Richlawn for the respective farms. Each farm received an experimental treatment comprised of 100 lb. of N per acre from the growers' preferred N fertilizer, and an additional amount of N from cyanobacteria, approximately 5.5 and 8 lb. per acre at the different farms. One of the farms had a third treatment group of 75 lb. of N per acre with approximately 5.5 lb. of N per acre from cyanobacteria as well. Characteristics yield weight, branch length, trunk cross sectional area, and mid-season leaf tissue nutrient analysis were measured. A hard freezing event in January, heavy pest pressure from green peach aphid, and higher than optimal N levels in leaf tissue which resulted in large variability in yield data. As a result of variability in data and N levels applied, significant differences between treatment and control groups were not found relative to farm. The application of cyanobacteria through a nearly identical irrigation system demonstrated how cyanobacteria raised on farm could be applied by orchardists without the need for extra equipment. Future research will include the investigation of techniques to increase N fixation rate and predictability with cyanobacteria. Additionally, a treatment will be added to the study to investigate differences between plots which are having cyanobacteria applied for a second season, and those for whom it is the first season of cyanobacteria application.

INTRODUCTION

Nitrogen (N) is the nutrient needed in the highest amounts by food crops. Nitrogen is a key component in amino acids which make up all proteins within plants. Although nitrogen gas (N₂) makes up nearly 80% of the Earth's atmosphere, it is unavailable to plants in its gaseous state. Production, transportation, and use of N fertilizers account for 75% of the carbon footprint of crop production (Smil, 2000; Vlek et al., 2004; Patel, 2008; Khan and Hanjra, 2009; Gan et al., 2012), due to the large amounts of natural gas needed to attain high pressures and temperatures, the transportation from the fertilizer plant to the farm, the application of fertilizers to crops, and losses of N₂O from the field. According to a recent survey conducted by our research group at CSU, organic fruit producers often use manure, compost, fish meal, or chicken meal as a source of nitrogen, which all have relatively high carbon to nitrogen ratios (C:N). Low N percentages of organic fertilizers results in large quantities of organic fertilizers being transported to the farm.

Cyanobacteria are photosynthetic, N fixing bacteria which use an enzyme, nitrogenase, to convert N₂ into ammonia. Ammonia is converted into ammonium (NH₄) which is a form that plants can use. Cyanobacteria are commonly found in a diversity of soils and water bodies, and are an important contributor of N in natural ecosystems worldwide. Cyanobacteria can be cultured in a laboratory, and grown on-farm in specialized ponds called raceways. In a raceway, water is circulated via a paddlewheel driven by an electric motor which allows carbon dioxide gas, necessary for cyanobacterial photosynthesis, to be integrated into the water. Once a growing cycle is complete, roughly two weeks, the cyanobacteria can be applied as a liquid biofertilizer through irrigation systems (our research group has demonstrated this fertigation process in drip irrigation systems).

Our objectives are to determine whether there is an effect of cyanobacterial biofertilizer on peaches in addition to traditional organic nitrogen sources and to evaluate whether cyanobacterial liquid biofertilizer can be applied directly through growers' existing micro-sprinkler irrigation systems.

METHODS

Cyanobacterial biofertilizer (*Anabaena* spp.) was grown on-farm and applied to two organic peach orchards near Hotchkiss, CO. The cyanobacteria were grown, using organic nutrient media (Barminski, 2014), in 6' x 18' and 6' x 24' raceway ponds with a 10 inch depth. The cultures were grown for periods of two weeks before application. Throughout the growing period, the growing conditions such as temperature and pH were monitored using a handheld pH meter (Hanna Instruments, Woonsocket, RI). Before each application was made, the N concentration of the biofertilizer was analyzed using a Hach DR 3900 spectrophotometer (Hach, Loveland, CO).

Application of the biofertilizer was made directly from the raceways via a sump pump into a separate irrigation system which ran parallel to the growers' irrigation systems. The biofertilizer irrigation system utilized identical micro-sprinkler emitters at the same spacing as the growers' existing system. Ninety percent of biofertilizer was applied every two weeks, leaving a remainder of 10 percent in the raceway pond. An equivalent amount of tap water was then added to the raceway, along with a proportionate amount of organic nutrient media, and allowed to grow for another 2 week period. Through the fertilizer application period of May 28th- August 1st, Farm A received a total of 1.28 gallons per square foot of the biofertilizer and Farm B received a total of 1.65 gallons per square foot, on treatment plots.

Experimental treatments were randomly assigned to plots (5 trees/plot) using a Randomized Complete Block Design with five replications. At farm A, there were 2 treatments: a control

treatment of 100 lbs N/acre from True Organic 12-3-0 (Spreckels, CA) the grower's choice organic N fertilizer, and 100 lbs N/acre from True Organic fertilizer along with 25 lbs N/acre from the cyanobacteria biofertilizer. At farm B, 3 treatments were assigned: a control treatment of 100 lbs N/acre from Richlawn 5-3-2 (Platteville, CO) the grower's choice organic N fertilizer, a treatment of 100 lbs N/acre of Richlawn in addition to 25 lb N/acre from cyanobacterial biofertilizer, and a third treatment of 75 lb N/acre of True Organic in addition to 25 lbs N/acre from cyanobacteria biofertilizer. Due to unforeseen N fixation rates, the actual N application rates for cyanobacterial biofertilizer applications totaled 8 lb N/acre at farm A, and 5.5 lb N/acre at farm B. The peach variety was Suncrest, the trees on Farm A were planted in 2008, and the trees on Farm B were planted in 1999. The soil type on farm A was Agua Fria Stony Loam, and the soil type on Farm B was Mesa Loam (<http://websoilsurvey.nrcs.usda.gov>). The planting spacing was 4' between trees and 15' between rows.

Measurements were taken from the three interior trees in every plot, leaving the two outside trees to act as buffer zones. First year branch growth was sampled on four branches per measured tree. Branches were selected of relatively similar size on May 27th, and for similar height, being 5 to 6 feet above the orchard floor. One branch was selected from each quadrant of the tree North, East, South, and West at farm A, and Northeast, Southeast, Northwest, Southwest at farm B. Measurements were taken biweekly by placing a ruler at the crotch of the branch, and measuring to the tip of the apical meristem.

Trunk cross sectional area (TCSA) measurements were taken on 4/26/2014 before biofertilizer application began, and after the trees had entered dormancy on 11/28/2014. Trunk circumference measurements were taken 8 inches above the orchard floor, and then converted to TCSA through the following equation: $TCSA = (\text{Trunk circumference} / 2\pi)^2 \times \pi$.

Sampling for midseason leaf tissue nutrient analysis was done as per instructions given by A & L West Labs (www.al-labs-west.com/). On 7/14/14 basal and midshoot leaves were selected from current year shoots, with petioles attached. Leaves were selected on branches which were 5' to 6' above the orchard floor. Two leaves from each compass direction were taken from each measured tree, for a total of 24 leaves per plot. Samples were then sent to A & L West Labs for analysis, and analyzed for nutrient concentrations.

Peaches were harvested on 8/28/2014 on farm A, and on 8/20/2014 and 8/27/2014 on farm B as the peaches approached the growers' desired ripeness. Total fruit count, and total yield weight were measured for each plot. Fruit circumference was measured on a randomly selected subsample of 10 peaches per plot, by using a measuring tape and measuring the circumference at the "equator" of the fruit with the stem being oriented vertically.

RESULTS AND DISCUSSION

First year branch growth measurements did not differ between treatments. Midseason leaf tissue nutrient analysis did not differ between treatments on the same farm, but did differ between farms (Table 1). Optimum nitrogen concentration for peach leaf tissue taken from April to May is 2.6-3.0% (La Rue 1989); therefore, leaf N values on Farm A are excessive, while those on Farm B are in the optimum range. Optimum zinc and manganese levels are in the optimum range of greater than 20 mg/kg (LaRue 1989).

Table 1. Midseason leaf tissue analysis results for Nitrogen, Zinc, and Manganese. Treatment T100 is 100 lbs N/acre from True Organic 12-3-0 fertilizer, T100+C25 is 100 lbs N/acre from True Organic fertilizer with 25 lbs N/acre from cyanobacterial biofertilizer, R100 is 100 lbs N/acre from Richlawn 5-3-2 fertilizer, R100 is 100 lbs N/acre from Richlawn fertilizer with 25 lbs N/acre from cyanobacterial biofertilizer, R75 is 75 lbs N/acre from Richlawn fertilizer with 25 lbs of N/acre from cyanobacterial biofertilizer. No significant differences were found for nutrient concentrations among treatments within farm ($\alpha=.05$).

Treatment	Leaf Tissue Nutrient Concentrations		
	Nitrogen ---%---	Zinc -----mg/kg-----	Manganese
Farm A			
T100	3.506	26.4	29.8
T100+C25	3.490	41.2	29.4
Farm B			
R100	2.768	29.2	55.4
R100+C25	2.756	30.8	60.0
R75+C25	2.706	29.8	56.4

TCSA measurements did not differ in terms of initial TSCA or final TSCA, when comparing treatments within farms. There were also no significant differences in yield between treatments (Table 2).

Table 2. Average yield and Standard deviation by treatment. No significant differences were found among yields within farm ($\alpha=.05$)

Treatment	Leaf Tissue Nutrient Concentrations	
	Avg. Plot Yield	Plot Yield Standard Deviation
-----Lbs-----		
Farm A		
T100	55.52	17.54
T100+C25	55.27	25.46
Farm B		
R100	88.69	13.39
R100+C25	127.25	10.78
R75+C25	127.19	17.49

The lack of significant differences between treatments likely had to do with several factors including: lower than expected nitrogen fixation by the cyanobacteria, high variability in the data, and relatively high N levels already present within the plants (especially on Farm A). Because the target N from the biofertilizer rate was not reached, it is not surprising that there would not be any significant difference. However, mid-season leaf tissue analysis indicated that there were already sufficient N levels present within the leaves, meaning adequate N was already available for the trees regardless of the additional N.

There was high variability in the data, which suggests that either different sampling methods should be used or higher replication is needed, especially for first year branch growth measurements. Variability also likely came from pest and freeze damage which were both very high at Farm A, and was responsible for massive yield losses in most of the plots at that farm.

It remains unknown why the N fixation rates were lower than those that have been achieved elsewhere with a similar culturing system. Many different factors may have been responsible for this issue including: high dissolved oxygen levels in raceways, contamination of antagonistic organisms of some kind, inadequate nutrient availability to cyanobacteria, and other possible factors. These factors will be investigated during the off-season to ensure the system is highly productive and consistent in the second experimental year.

It is difficult for conclusions to be drawn as to the effectiveness of cyanobacteria on peach trees, given the lower than expected rates which were applied. This study did however demonstrate that the biofertilizer can be applied through standard grower micro-sprinkler irrigation systems.

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