THESIS

EFFECTS OF CYANOBACTERIAL FERTILIZER, COMMONLY-USED ORGANIC FERTILIZERS, AND PLANT GROWTH REGULATORS ON YIELD AND GROWTH CHARACTERISTICS OF CARROTS (*DAUCUS CAROTA VAR. SATIVUS*), CUCUMBERS (*CUCUMIS SATIVUS*), AND BELL PEPPERS (*CAPSICUM ANNUUM*)

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ABSTRACT

EFFECTS OF CYANOBACTERIAL FERTILIZER, COMMONLY-USED ORGANIC FERTILIZERS, AND PLANT GROWTH REGULATORS ON YIELD AND GROWTH CHARACTERISTICS OF CARROTS (*DAUCUS CAROTA VAR. SATIVUS*), CUCUMBERS (*CUCUMIS SATIVUS*), AND BELL PEPPERS (*CAPSICUM ANNUUM*)

Nitrogen (N) is arguably the most important agricultural nutrient. More money and resources are spent on N management in agricultural systems than any other nutrient. Producing N fertilizer for agricultural use accounts for more than half of the carbon footprint of crop production. Nitrogen plays a crucial role in plant growth, and adding N fertilizers to agricultural systems can lead to noticeable increases in productivity. Nitrogen fertilizers commonly used in organic production are often energy intensive to produce and expensive to transport. Cyanobacteria fertilizer (cyano-fertilizer) produced on-farm could decrease fertilizer impacts on the environment as well as reduce production costs for organic farmers. In addition, cyano-fertilizer may perform similarly to products marketed to increase production via plant growth hormones such as seaweed extract, which is shipped all over the world from coastal regions. The effects of common organic fertilizers as well as organic liquid cyano-fertilizer on carrot (*Daucus carota var. sativus*) and cucumber (*Cucumis sativus*) growth and yield characteristics were tested during field experiments at the Horticulture Field Research Center in Fort Collins, CO in 2014 and 2015. Bell peppers (*Capsicum annuum*) were grown in a greenhouse experiment in 2015 at the Colorado State University Plant Growth Facility. Cyano-fertilizer was produced and

evaluated in this study to compare effects of farm-grown cyano-fertilizer and commonly-used organic fertilizers.

The purpose of this study was to identify fertilizer and foliar seaweed application effects on yield, stress, and growth characteristics of all three plant species. In all experiments, hydrolyzed and non-hydrolyzed fish fertilizers, and cyano-fertilizer treatments were applied at prescribed N rates throughout the growth period approximately every 10 days. Control treatments received no supplemental N. Each treatment, including the control, was repeated with the addition of two forms of concentrated organic seaweed extract applied foliarly. Neptune's Harvest and Seacom PGR brand seaweeds were used for their lack of N content. Seaweeds were applied at the manufacturers' recommended rates. Phytohormones were detected in all N fertilizers and in the PGR seaweed. No phytohormones were detected in the Neptune's Harvest seaweed. In 2014, carrot length and yield were increased by the addition of cyano-fertilizer compared to the unfertilized control. All fertilizers increased post-season soil N compared to the control. Nitrogen fertilizers increased carrot leaf tissue Mg concentrations compared to the control. Nitrogen fertilizers and foliar seaweed influenced the number of carrots with deformities, and a significant interaction between N fertilizers and seaweed with regard to stress indicated a stress response to the addition of both fish fertilizer and a foliar seaweed application. In 2015, cyano-fertilizer produced a higher carrot yield than hydrolyzed fish fertilizer. Nitrogen fertilizers impacted the total number of cucumbers harvested as well as total cucumber yield, but the results were not consistent across years. The majority of significant differences occurred in the pepper study. Nitrogen fertilizer had an effect on leaf tissue nutrient concentrations as well as phytohormone content. Nitrogen fertilizer also impacted flower death and leaf abscission as well as plant stress. Foliarly applied seaweed treatments had very little significant influence in the carrot or cucumber field studies, but did have an effect on pepper shape and color (crop quality). Pepper yield was impacted by the addition of N fertilizers. Foliar seaweed impacted pepper branching behavior as well as fruit color and shape. Based on these experiments, it can be concluded that cyano-fertilizer can be used as a N source in place of commonly-used organic fertilizers. With regards to plant growth characteristics, it is unclear that any of the products applied consistently impacted plant growth characteristics in a way that improved yield or quality.

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INTRODUCTION

Nitrogen (N) is a major component of almost all biological molecules in plants including amino acids, enzymes, nucleic acids, chlorophyll, and more. N is essential for using carbohydrates and taking up other nutrients (Brady and Weil, 2007). Plants cannot use atmospheric N, and, therefore, the plant's needs must be met entirely by the soil. Most N exists in organic forms in the soil, until being broken down by soil microbes via mineralization into plant available inorganic N or small organic compounds. To increase yields, farmers supplement their soil N levels with fertilizers applied to the soil before and during the growing season. N fertilizers come in many forms: solid, liquid, gas, and can be inorganic or organic in nature. There are many precise inorganic fertilizers available in conventional agriculture, but consumers have become more concerned with how their food is produced and its impact on the environment. Simultaneously, growers are trying to produce more food with less environmental impact in order to continue to produce food sustainably.

Nitrogen management on organic farms can be difficult due to the nature of organically-approved fertilizers. Organic farmers use compost, manure, legume cover crops, dried organic meals such as feather and blood meal, or liquid fertilizers such as fish emulsion to increase crop productivity. Additionally, there are many specialty products on the market containing plant growth regulators, or phytohormones, intended to impact plant growth characteristics which could increase yield. Many products also contain soil microbes which are designed to enhance the breakdown of organic matter, releasing plant available N. The use of these fertilizers is often imperfect; liquid fertilizers and meals are energy intensive to produce and ship, and yield impacts of specialty products are often not conclusive (Hemphill, 1981; Hamza and Suggars, 2001; Aliyu et al., 2011). In organic farming systems, the most common forms of N are largely organic matter, such as composted manure. This makes N management difficult, as organic N mineralization rates are difficult to predict and control (Schütt et al., 2013; Sierra et al., 2015). To have predictability of N inputs, organic farmers often turn to fertilizers such as liquid fish fertilizer to supply their crops with supplemental N mid-season. Purchasing and transporting fertilizers such as fish fertilizer can be costly and can have a large carbon footprint, contrary to the aims of organic agriculture.

Fish fertilizer, as it is so common in organic practices, was used in this study. Two common brands were examined: Alaska Fish Fertilizer (Walnut Creek, CA) and Neptune's Harvest Hydrolyzed Fish Fertilizer (Gloucester, MA). Alaska Fish Fertilizer is non-hydrolyzed. In fertilizer manufacturing, hydrolyzed generally means that the whole fish is cold processed in water and is broken down using naturally occurring enzymes, whereas non-hydrolyzed is thought to be heat processed or cooked down to concentrate the nutrients. One of each type of fertilizer was used to better understand the impacts of all types of fish fertilizers on vegetable yield and quality. The benefits and possible negative consequences of using each type of fish fertilizer were compared. Cyano-fertilizer was also used in this study as an N source. Cyano-fertilizer is an on-farm, grower-grown N source which contains cyanobacteria. The fertilizers used in this study and their grades are summarized in Table 1.

Cyanobacteria are an omnipresent phylum of bacteria capable of photosynthesis and atmospheric N_2 fixation. Creating an environment that fosters cyanobacterial growth results in a liquid fertilizer (cyano-fertilizer), which can be used as an organic N source. Cyano-fertilizer can be applied to crops through fertigation. Once in the soil, microbes mineralize the organic N from the cyanobacteria for plant use. By growing cyano-fertilizer organically on-farm, organic farmers can harness the N gathering power of these prokaryotes and potentially decrease the cost and carbon footprint of purchasing and transporting traditional organic fertilizers. While cyano-fertilizer has a lower N concentration than fish fertilizers, fish fertilizers are usually diluted prior to application, and both can be applied multiple times throughout the season. Composts and manures, on the other hand, are usually applied pre-plant only. Additionally, in a greenhouse study conducted by Sukor (2013), N from dried cyanobacteria had a 6% higher mineralization rate than composted manure. In the same study, Sukor (2013) found that cyano-fertilizer increased lettuce yield by 58% compared to composted manure applied at the same N rate.

Studies have found that cyanobacteria are capable of producing an elaborate array of secondary compounds (Yadav et al., 2011). Preliminary data shows that phytohormones were present in cyano-fertilizer grown at CSU in 2014 as shown in Table 2. These results agree with the review by Yadav et al. (2011) describing the ability of cyanobacteria to produce auxins, cytokinins, and abscisic acid. Phytohormones are marketed by manufacturers to stimulate plant growth when applied exogenously. Organic farmers can purchase products such as organic liquid seaweed extract to apply exogenous phytohormones to crop foliage or as a soil soak. Seaweed products are best known for their auxin and cytokinin contents. Endogenous auxins and cytokinins are responsible for cell division and root and shoot elongation, respectively (Hamza and Suggars, 2001). Salicylic acid, also found in cyano-fertilizer in this study, is known to play a role in plant response to abiotic stress (Kumar, 2014). A newly discovered plant hormone, strigolactone, interacts closely with auxin within plants and has been connected with branching behavior in peas (Gomez-Roldan et al., 2008). Changing branching behavior of food crops could increase yield and provide better weed control for organic farmers. The potential impact of applying plant growth regulators for increased yield or plant health is still unknown. Hamza and

Suggars (2001) found that exogenously applied phytohormones improved the quality of turf grass only when used in conjunction with N fertilizers, but the combination led to higher quality grasses than N fertilizer alone. Hemphill (1981) applied cytokinin to celery, strawberries, carrots, tomatoes, and potatoes, and found that only tomatoes produced greater yield in response to cytokinin. However, Aliyu et al. (2011) found that pre-bloom application of phytohormones increased flowering and fruiting in cashews. Although phytohormones in cyano-fertilizer are relatively dilute in comparison to the full strength fish fertilizers, fish fertilizers are diluted when applied, while the cyano-fertilizer is applied at full strength. When all fertilizers are applied at equal N rates, more phytohormones are applied to crops from cyano-fertilizer than fish fertilizers or liquid seaweed products applied at the manufacturers' recommended rates (Table 2).

Two liquid seaweed extracts were used in this study: Seacom PGR (Freeport, ME) and Neptune's Harvest Seaweed Plant Food (Gloucester, MA). Seacom PGR was originally chosen to be the only foliar seaweed treatment, but due to difficulties in proving that the product was certified organic, the foliar seaweed had to be switched for the second year of field studies. Both seaweeds were tested in the greenhouse experiment to determine if they performed similarly. Both seaweeds were chosen because the seaweed was cold processed to retain integrity of biological molecules, and because they contained no N, reducing interference with the effects of N fertilizers.

There is an opportunity to improve the organic fertilizer supply. To make progress towards the overarching goals of organic agriculture, the carbon footprint of organic fertilizer use should be decreased. Additionally, with contradictory findings surrounding the effectiveness of organic products, this study was designed to shed light on commonly-used organic products and new ones that may reduce the carbon footprint of organic agriculture.

The overarching objectives of this study were to 1) evaluate cyano-fertilizer compared to traditional organic fertilizers in providing adequate N to increase yield in carrots, cucumbers, and bell peppers; and 2) characterize the impact of applying exogenous phytohormones in cyano-fertilizer as compared to products such as liquid seaweed on the same crops.

There were 2 hypotheses associated with these objectives. 1) It was hypothesized that cyano-fertilizer would perform as well as, or better than, traditional organic fertilizers as an N source for increasing yield. 2) It was hypothesized that growth characteristics such as deformities, branching, flowering, leaf to stem ratios, and others would be influenced by phytohormones.

CHAPTER 1

EFFECT OF LIQUID ORGANIC FERTILIZERS AND SEAWEED EXTRACT ON CARROT (*DAUCUS CAROTA VAR. SATIVUS*) AND CUCUMBER (*CUCUMIS SATIVUS*) GROWTH CHARACTERISTICS

SUMMARY

Effects of common organic fertilizers as well as organic cyano-fertilizer on carrot (*Daucus carota var. sativus*) and cucumber (*Cucumis sativus*) growth and yield characteristics were tested during field experiments at the Horticulture Field Research Center in Fort Collins, CO in 2014 and 2015. The purpose of this study was to identify fertilizer and foliar seaweed application effects on yield, stress, and growth characteristics of carrot tops and roots as well as cucumber fruits and vines. Hydrolyzed and non-hydrolyzed fish fertilizer and cyano-fertilizer treatments were applied at prescribed N rates and distributed throughout the growth period approximately every 10 days. Control treatments received no supplemental N. Each treatment, including the control, was repeated with the addition of concentrated organic seaweed extract applied foliarly at the manufacturer's recommended rate.

When comparing N fertilizer treatment effects on carrots, no significant differences were detected in plant height, wet and dry weight of plant materials, leaf to stem ratios, total biomass, or total water content. Carrot leaf tissue sample analysis reported comparable leaf concentrations of N, K, S, Ca, Zn, Fe, Mn, Cu, B, and Mo. All treatments produced carrots with similar incidence of cracks or splits as well as branching in the roots. All treatments provided similar levels of post-harvest soil NH_4^+-N. In 2014, cyano-fertilizer and non-hydrolyzed fish fertilizer yielded greater than the control for carrots. In 2015, cyano-fertilizer produced a greater carrot

yield than hydrolyzed fish fertilizer, but all treatments were comparable to the control. In 2014, cyano-fertilizer reduced root knobs and increased carrot length and diameter. In the same year, cyano-fertilizer and hydrolyzed fish fertilizer reduced the occurrence of under-developed carrot roots. Foliar seaweed applications also contributed to decreasing these root deformities; based on F-test statistics, foliar seaweed had more influence on carrot root knobs than the N fertilizers.

For cucumbers, all treatments produced cucumber vines with similar stress levels, wet weights, dry biomass, and water contents. Although there were significant impacts on yield each year, the impacts were not consistent from year-to-year. In 2014, the non-hydrolyzed fish fertilizer treatment produced a greater yield and higher number of cucumbers harvested compared to cyano-fertilizer, though all treatments were comparable to the control. In 2015, the non-hydrolyzed fish fertilizer produced fewer cucumbers than the control and the cyano-fertilizer, and both fish fertilizers produced lower yield than the control. Also in 2015, cucumber plants receiving foliar seaweed produced cucumbers with an average diameter of 6.23 cm, wider than the average diameter of those that did not receive the foliar treatment (5.96 cm) at the second harvest. Foliar seaweed applications increased average cucumber length (23.5 cm) compared to plants receiving no foliar seaweed (22.8 cm) measured at the second harvest ($p<0.07$).

While most treatments performed similarly to the control, when significant difference occurred, cyano-fertilizer performed similarly to other fertilizers, suggesting that cyano-fertilizer could be used in place of commonly-used organic N fertilizers. Additionally, the foliar seaweed treatment may be more effective at a higher rate, but did not seem to improve growth yield or quality except for reduction of carrot root knobs in 2014 and increased cucumber size in one of two harvests in 2015.

INTRODUCTION

Carrots (*Daucus carota var. sativus*) are a member of the Umbelliferae family, which also includes celery, parsnip, parsley, dill, caraway, anise, coriander, and fennel (Kelley et al., 2009). Carrots are biennial plants with a distinct taproot. The stems of the carrot top support compound, alternating leaves, and the stems can grow from 60 to 200 cm in height. Roots can range from 1 to 10 cm in diameter, and from 5 to 50 cm in length, with 10 to 25 cm in length being the norm (Rubatzky et al., 1999).

Young carrots are not tolerant to extreme temperatures, and in all stages, persistent hot weather can cause high levels of terpinoids which affect the flavor, and can lead to stunted root growth (Kelley et al., 2009). Adequate and consistent moisture is best to avoid root cracking, usually caused by growth flushes (Kelley et al., 2009). Carrots have a “medium” N requirement, and do well with 100 to 135 kg N ha^{-1} (Kelley et al., 2009). Timing of N application is of equal importance, as carrots must be “spoon-fed” N in order to prevent growth spurts and cracking (Kelley et al., 2009). Westerveld et al. (2006) found that carrots can take up 72 – 250 kg ha^{-1} depending on soil conditions. On a mineral soil in a dry year, net N removal for carrots was 72-81 kg ha^{-1}, which is lower than previously sited values of 150 kg ha^{-1} in Finland and 178 kg ha^{-1} in Michigan (Westerveld et al., 2006). In the same study, carrot yields did not respond to an N rate of 110 kg ha^{-1} on mineral soils, and carrots usually had sufficient soil N without fertilizer application (Westerveld et al., 2006). Conversely, Hochmuth et al. (1999) found that yield and quality parameters were maximized at 140 kg ha^{-1} and 160 kg ha^{-1} depending on planting date. The target N of 135 kg ha^{-1} in this study fits well into the middle of these published carrot N use values.

The most common disease affecting carrot marketability is the root knot nematode caused by *Meloidogne sp.*, and injuries to carrots can be entry ways for other diseases. Pythium blight is another common disease that can cause underdeveloped roots (Kelley et al., 2009). Many companies that produce or sell liquid seaweed extracts claim that foliarly applied seaweed fertilizers can increase crop resistance to nematode, pests, and other diseases. Carrot branching can be caused by pests, blights, and soil conditions. All of these deformities are common and can seriously impact the marketability of a carrot crop.

Approximately half of all carrots produced in the United States in 2009 came from California, with states such as Texas, Washington, Michigan and Wisconsin contributing most of the rest (USDA ERS, 2011). The USDA reports that on March 2^{nd}, 2016, the average wholesale organic carrot price (11.3 or 22.6 kg crates or sacks or loose carrots) in the US was $0.90 per 0.45 kg. Compared to conventional, at $0.40 per 0.45 kg, growing organically can more than double the value of carrots. The market was “about steady” in most cities on March 2^{nd} 2016, with some cities reporting “steady” markets (USDA AMS, 2016).

Cucumber (*Cucumis sativus*) is a member of the Cucurbitaceae family, also known as the gourd family. The cucumber plant has long vines with cylindrical fruits and comes in three main types: slicing, pickling, and burpless. Within these categories, cucumber varieties come in various shapes and sizes. Most cucumber cultivars require pollination to produce fruit. Cucumbers grow best in soil temperatures above 15.5 ^{o}C (CSU Plantalk, 2016). Cucumbers are sensitive to extreme temperatures and can be killed by a slight frost. Cucumbers have a shallow root system, and require adequate irrigation, but will perform better under deep, infrequent irrigation than frequent shallow irrigation unless the weather is hot and dry, as is typical of Colorado summers.

Recommendations are to provide cucumbers with "moderate" fertilization, 89 to 112 kg N ha^{-1} pre-plant (NCSU Extension, 2000). However, Van Eerd and O'Reilly (2009) found that cucumbers showed little to no response to added N in soils that contained an average of 81 kg ha^{-1} of pre-plant inorganic N, arguing that cucurbits often have little to no N response and that 81 kg ha^{-1} was sufficient. The most common diseases impacting cucumber vines are bacterial wilt and powdery mildew (Clemson Extension, 2015). Angular leaf spot is also common, and can be mitigated by applying N to encourage new growth. Blossom end rot is the most common disease affecting the fruit; and may be the result of a lack of available soil calcium, although there is uncertainty about the cause. Several insects can damage cucumbers such as pickleworm, squash and cucumber beetles, spider mites, squash vine borers, and squash bugs. Disease and pest control methods vary depending on conditions, but in general, rotating cucumbers with non-cucurbits yearly is recommended as the best form of pest and disease control.

The market for cucumbers on March 3rd, 2016 was reported in all U.S. cities as about steady, steady, or higher (USDA AMS, 2016). The price for 12 film-wrapped, long, seedless organic cucumbers was $36.00, compared to conventional at $11.77 (USDA AMS, 2016). Growing cucumbers organically can more than triple the value of cucumbers.

In this experiment, carrots and cucumbers were grown in Fort Collins, CO in the 2014 and 2015 seasons to evaluate the impact of various organic fertilizers and foliar seaweed extracts on vegetable growth characteristics and marketability. If farm-grown cyano-fertilizer is able to produce as much yield with greater or equal quality to the other organic fertilizers and/or the control, it may be a less carbon intensive way to grow vegetables organically. Producers and consumers of organic food may be interested in decreasing the carbon footprint of food production while maintaining or improving yield, as such practices align well with the goals of organic agriculture. Additionally, the benefits of adding foliar seaweed treatments to organic vegetable regimens remains controversial; therefore, the potential impacts were examined.

MATERIALS AND METHODS

STUDY SITE

Field experiments were conducted during the 2014 and 2015 growing seasons on certified organic land at the Colorado State University Horticultural Research Center (4300 E County Road 50, 80524) in Fort Collins, CO. The soil in this location was described as a fine, smectitic, mesic, Aridic Argiustoll of the Nunn series (USDA NRCS, 1980). Pre-season soil samples were taken to a depth of 58.8 cm. Soil analysis reported soil pH to be, on average, 8.1. The starting organic matter at the study site was 2.7%. Weather data was gathered for both growing seasons, which is considered to be the time period between May 1st and September 30th. The average maximum temperature was 26^{o}C in both 2014 and 2015. The average minimum temperature was 18 ^{o}C in 2014 and 17 ^{o}C in 2015. There were 2.1 cm and 6.1 cm of rain in 2014 and 2015, respectively. Both field experiments took place on certified organic land, but the location of the plot was changed slightly to avoid residual N influence from year-to-year. Pre-season inorganic soil N analyses were performed by Ward Laboratories Inc. in Kearney, NE in 2014 and the Colorado State University Soil, Water, Plant Testing Laboratory (Fort Collins, CO) in 2015. Samples were taken from each of the eight study blocks to a depth of 58.8 cm. Soils were extracted with 2 M KCl, and NO_3^--N and NH_4^+-N were measured by automated colorimetry (Table 3). Sample results were averaged to obtain the pre-season soil inorganic N value used in determining N prescriptions.

EXPERIMENTAL DESIGN

The carrot and cucumber studies were designed as randomized complete block designs with a 4x2 factorial of four soil treatments with and without foliar seaweed for a total of 8 treatments per replication (Table 1). Four replications of the 4x2 factorial were randomly assigned within the plot, for a total of 32 rows for both carrots and cucumbers. Before planting in both years, a drip irrigation system was installed to supply water and N fertilizers. The system utilized two 24.38 m headers running length wise for each crop, outlining the plot with drip tape rows in between. A ball valve was installed at each end of the drip tape where it joined with the headers for the purpose of selectively closing rows to facilitate fertigation through the irrigation system. Large ball valves were installed at the ends of the headers so that low-pressure, clean water could be flushed through the lines between treatments to minimize cross contamination. The drip tape used was John Deere 15 mil, 20.3 cm spaced emitters, 2.5 L min^{-1} per 30.5 cm. All weeding was done by hand, and no herbicides, fungicides, or pesticides were applied.

TREATMENTS

The target N rate for carrots in both years was 135 kg N ha^{-1}, and the target total N rate for cucumbers in both years was 120 kg N ha^{-1}. N fertilizer prescriptions were assigned after pre-season soil testing. The three N fertilizers used in this experiment were: cyano-fertilizer, hydrolyzed fish fertilizer, and non-hydrolyzed fish fertilizer. The cyano-fertilizer was grown on farm using methods described by Barminski et al. (2016). Neptune's Harvest hydrolyzed fish fertilizer and Alaska non-hydrolyzed fish fertilizer were purchased from Neptune's Harvest (Gloucester, MA) and Fort Collins Nursery (Fort Collins, CO), respectively. The three fertilizers varied in N concentration and were applied at equal N rates. The application rates differed slightly for carrots and cucumbers; dates are described in Table 4 and Table 5, respectively. N fertilizers were injected into a drip irrigation system (described below) and applied directly to the soil. The control group received no N fertilizer, and water supplied through the N treatments was calculated and supplied to the control rows the next day to equalize water application.

Organic seaweed extract was purchased from Johnny's Selected Seeds (Winslow, ME) in 2014 and from Neptune's Harvest (Gloucester, MA) in 2015. The seaweed extract was applied foliarly using a backpack sprayer.

FERTIGATION

N fertilizers were applied directly through the drip irrigation system utilizing the row valves to control application to the appropriate rows. The cyano-fertilizer was grown in a production raceway on farm and applied full strength using a sump pump placed in the production raceway. Fish fertilizers were diluted into a livestock watering tank to match cyano-fertilizer N concentration for each application, and also applied with a sump pump. The N content of the cyano-fertilizer was measured the day of fertigation using a Hach DR3900 Benchtop Spectrophotometer (Loveland, CO) to measure Total Kjeldahl Nitrogen. The sump pump had to be routinely cleaned to remove solids trapped in the pump chamber.

IRRIGATION

Automatic irrigation was used to irrigate the rows for 45 minutes, 5 days a week to apply 10.5 mm day^{-1} in each row. Irrigation was scheduled to start 5 days a week so that one day could be used for fertilization and the last day of each week could be used to apply water to the control and fish fertilizer rows to match cyano-fertilizer applications. Applied irrigation water for carrots totaled 821 mm and 610 mm in 2014 and 2015, respectively. Applied irrigation water for cucumbers was 497 mm in 2014 and 700 mm in 2015.

CARROT VARIETY AND PLANTING

Organic “Nectar” (F1) carrot (*Daucus carota var. sativus*) seeds were purchased from Johnny's Selected Seeds (Winslow, ME). This variety is said to be uniform and flavorful, 17.7-20.3 cm in length, with medium-tall tops that hold up well to leaf blight.

The carrots were double planted (sub-rows) in a 3 m length of row, with 7.6 cm spacing between plants and 76 cm centers between rows. Each sub-row was planted 7.6 cm away from the drip tape center. After emergence, the carrots were thinned to contain approximately 40 carrots per sub-row, for a total of 80 carrots surrounding each drip tape. In order to minimize edge effects, the center 10 carrots (5 per sub-row) were flagged for measurements as representatives of the row.

CUCUMBER VARIETY AND PLANTING

Organic “Corinto” (F1) cucumber seeds were purchased from Johnny's Selected Seeds (Winslow, ME). This variety is said to be productive and uniform, with cucumbers 17.7-20.3 cm in length and intermediate resistance to cucumber diseases.

Cucumbers were direct seeded in 2014 and transplanted into the field in 2015.Ten cucumber plants were planted in each of the 32 single rows, 8 cm away from the center of the drip tape. All 10 cucumbers were used for measurements. In 2014, the rows were each 3 m long, but were extended to 3.7 m long in 2015.

CARROT MEASUREMENTS

For both seasons, weekly measurements taken during the season included plant height and plant stress. Plant height was measured in cm with a tape measure to the tip of the tallest leaf in the carrot top. Plant stress was monitored using an original point-based scale. The scale ranged from 0 to 5 with 0 being a completely healthy plant, and 5 being a plant that is dead or beyond the permanent wilting point. An example of a stressed plant can be seen in Figure 1. This scale was created to be all encompassing; it included drought stress, rodent damage, insect infestations, and discoloration caused by nutrient deficiency or disease.

On the date of harvest, the center 10 carrots from each row were harvested, and the tops were cut off at the crown. The carrot roots were examined for marketability deformities. The number of individual carrots displaying branched roots, root knobs, cracks or splits, and underdeveloped root length were counted for each row (Figure 2). Carrot root length and circumference at the crown were measured with a tape measure. Circumference was converted to diameter for statistical analysis and reporting. All 10 carrots from each row were weighed together to obtain yield in kg ha^{-1}. All 10 carrot tops were weighed together while wet, then the leaves were stripped from the stems and petioles. Above ground biomass (wet) was compared to root mass in each row to obtain a root to shoot ratio. The wet leaves and stems were then weighed separately and dried at 49^{o}C for 3 days. The dried leaves and stems were re-weighed to obtain dry matter content of each. Wet and dry weights of leaves and stems were compared to determine a leaf to stem ratio by mass. Dried leaves were ground, and leaf tissue samples were analyzed for N, P, K, S, Ca, Mg, Zn, Fe, Mn, Cu, B, and Mo at Ward Laboratories Inc. in Kearney, NE utilizing ICP spectroscopy. After harvest, each row was soil sampled under the drip tape, and samples were analyzed for NO_3^--N and NH_4^+-N. The samples were taken to 45.7 cm using a Giddings soil sampling rig. The samples were air-dried, ground, and sieved to 2 mm. Inorganic N was extracted using a 1:10 soil to solution ratio in 2M KCl. In 2014, the extracts were analyzed with the Alpkem auto-analyzer at the CSU EcoCore Analytical Services in Fort Collins, CO. In 2015, the extracts were analyzed by the CSU Soil, Water, and Plant Testing Laboratory in Fort Collins, CO by automated colorimetry with a Lachat auto-analyzer.

CUCUMBER MEASUREMENTS

During both seasons, plant stress was measured weekly during the growing season using the point-based scale described above. On the day of harvest, all of the cucumbers harvested per row were weighed for a total row yield. Unmarketable or blemished cucumbers were discarded, and did not count toward the total produced cucumbers for each row (Figure 3). Length and circumference were measured for each cucumber, and averages were calculated per plot. Post-harvest, the cucumber plants were cut at the soil level, weighed, and placed in a dryer at 49ºC for 5 days. The dry weight, or biomass, was measured for each row. After harvest, each row was soil sampled under the drip tape, and samples were analyzed for NO_3^--N and NH_4^+-N. The samples were taken to 45.7 cm with a Giddings soil sampling rig and air dried, ground, and sieved with a 2 mm sieve. Inorganic N was extracted using a 1:10 soil to solution ratio in 2M KCl. In 2014, the extracts were analyzed with the Alpkem auto-analyzer at the CSU EcoCore Analytical Services in Fort Collins, CO. In 2015, the samples were analyzed by the CSU Soil, Water, and Plant Testing Laboratory in Fort Collins, CO in 2015 utilizing a Lachat auto-analyzer.

2014 CARROTS

Carrots were planted on May 20th, 2014 at a rate of approximately 500,000 seeds ha^{-1}. The seedlings emerged on June 1st, 2014. The rows were thinned to 80 carrots, and the center 10 plants were flagged on June 20th, 2014. Based on 2014 pre-season soil sampling, the target N application was 85 kg N ha^{-1} over the growing season to reach a total N of 135 kg N ha^{-1}. Due to weather challenges, only 68 kg N ha^{-1} were able to be applied before harvest. Fertigation occurred five times, approximately every two weeks (Table 4). PGR seaweed was applied foliarly with a backpack sprayer at the manufacturer's recommended rate of 1.2 L ha^{-1} three times over the season: immediately after appearance of seedlings, mid-growth, and 20 days before maturation (Table 6). Harvest occurred on September 6th, 2014, 109 days after planting.

<u>2014 CUCUMBERS</u>

On July 8th, 2014, cucumber seeds were seeded directly into the field at the study site at a rate of approximately 60,000 seeds ha^{-1}. Two seeds were planted into each hole. The holes were 30.4 cm apart and 8 cm away from the drip tape. The seedlings emerged on July 14th, 2014, and the cucumbers were thinned to contain 10 plants per row. Based on the preseason soil test, 76 kg N ha^{-1} were needed to achieve the total soil N target of 120 kg N ha^{-1}. The target was reached in 5 fertilizer applications (Table 5). Foliar seaweed extract was applied three times throughout the season: once at seedling emergence, and twice more at three week intervals. Foliar seaweed was applied at the manufacturer's rate of 1.2 L ha^{-1} for a total of 3.6 L ha^{-1}.

On September 12th, 2014, the cucumbers were harvested from the vines, 67 days after planting. All cucumbers for each row were bagged together, weighed, and counted. Individual cucumbers were then measured in length and circumference to obtain a row average. On the morning of September 13th, due to an early frost that killed the plants, the cucumber vines were cut at the soil level, weighed, and dried at 49°C for 6 days. Dried plants were reweighed to quantify above ground biomass and water content.

2015 CARROTS

Carrot seeds were planted on May 13th, 2015 at a rate of approximately 500,000 seeds ha^{1}; however, due to a persistently rainy month which provided 13.6 cm of rain and below average temperatures, the germination rate of the carrots was poor. Seeds were replanted on June 8th, 2015, and the seedlings emerged on June 28th, 2015. On July 3rd, 2015, the carrots were thinned to 80 carrots per row. Fertigation occurred seven times throughout the season. The target N application rate based on the preseason soil test was 81 kg N ha^{-1}. The target was reached using both of the fish fertilizers. However, due to challenges with a predatory microbe contamination, the cyanobacteria population was impacted. The cyano-fertilizer was only able to be applied at a rate of 28 kg N ha^{-1}. All 2015 data reflects the unadjusted values for effects of cyano-fertilizer (Table 4). Neptune's Harvest seaweed extract was applied with a backpack sprayer according to manufacturer's recommended dilution rate of 30 mL L^{-1}, with enough solution to coat all leaves of the plants in a foliar application. The seaweed was applied five times over the season: immediately after appearance of seedlings, three times during mid-growth, and a week before maturation (Table 6). The 10 center carrots from each row were harvested on August 28th, 2015, 81 days after the second planting.

2015 CUCUMBERS

On May 13th, 2015 cucumber seeds were planted into 72 cell plug trays filled with organic potting soil provided by the CSU Plant Growth Facilities. The trays were covered with plastic lids for rodent protection, and placed in a certified organic Quonset at the W.D. Holley Plant Environmental Research Center (PERC) Greenhouse at CSU. The cells were kept moist and warm during seed germination. The seedlings emerged on May 19th, 2015. On May 25th, 2015, 320 cucumber plants were transplanted into the field, 30.4 cm apart and 8 cm away from the drip tape at approximately 60,000 seeds ha^{-1}. Based on the preseason soil test, 61.7 kg N ha^{-1} were needed to reach the soil N target of 120 kg N ha^{-1}. Due to challenges with a predatory microbe invasion, the cyanobacteria population was impacted. No cyano-fertilizer was applied after July 23rd, 2015. Cyano-fertilizer was applied at 23.2 kg N ha^{-1}. The fish fertilizers were applied just below the target rate at 58.5 kg N ha^{-1} (Table 5). Seaweed extract was applied at the manufacturer's recommended dilution rate of 30 mL L^{-1}, with enough solution to coat all leaves of the plants in a foliar application. Seaweed extract was applied four times throughout the season; once after transplanting, and thrice more at two week intervals.

On July 26th, 2015 the cucumbers were harvested from the vines, 75 days after planting. All cucumbers for each row were bagged together, weighed, and counted. Individual cucumbers were then measured in length and circumference to obtain a row average. On August 14th, the cucumbers were harvested from the vines for a second time 94 days after planting, and the same measurements were repeated from the first harvest. On August 21st, 101 days after planting, the cucumber vines were cut at the soil level, weighed, and dried at 49^{o}C for 5 days. Dried plants were reweighed to quantify above ground biomass and water content.

STATISTICAL ANALYSIS

All statistics were performed using Statistical Analysis System 9.4 (Cary, NC). The procmixed statement was used, and the experimental design was run as a 4x2 factorial. Treatment and foliar seaweed applications were fixed effects, and block, or replicates, were treated as a random variable. There were serval factors that made it difficult to compare 2014 and 2015 seasons other than to look for patterns in results. The weather was very wet in 2015, whereas 2014 was drier. Additionally, the foliar seaweed used in the experiment had to be changed between seasons. For that reason, the years were analyzed separately. The slice statement was used to analyze effects of foliar seaweed extract. An adjusted F-test of fixed effects was performed using the REML method. Least square means were estimated with the lsmeans statement and compared with the pdiff statement. P-values <0.05 were considered significant in all cases.

RESULTS AND DISCUSSION

CARROTS

The ANOVA results (F-test statistics) for the 2014 and 2015 carrot studies are listed in Table 7 and Table 8, respectively. Only significant results are displayed in this table. All other variables were statistically similar among N fertilizers or between foliar seaweed applications.

The 2014 season presented more significant differences than the 2015 season. Among N fertilizers, no significant differences were detected in plant height, wet and dry weight of above-ground plant parts, leaf to stem ratios, total biomass, or water content. Leaf tissue sample analysis reported comparable leaf concentrations of N, P, K, S, Ca, Zn, Fe, Mn, Cu, B, and Mo. When comparing the marketability of the carrots, all treatments produced carrots with similar incidence of cracks or splits as well as branching in the roots.

The variable which was significant in both years was yield. In 2014, the cyano-fertilizer and the non-hydrolyzed fish fertilizer yielded greater than the control by 33.8 and 32.6 MT ha^{-1}, respectively (Figure 4). In 2015, the cyano-fertilizer produced a larger carrot yield than the hydrolyzed fish fertilizer, but all treatments were comparable to the control (Figure 4). Although there is no clear repeating pattern of differences, the data from the first year shows that the cyano-fertilizer performed as well as, or better than, commonly-used organic fertilizers and the unfertilized control with regard to yield. These results are consistent with findings by Hochmuth et al. (1999), in which carrot quality and yield were optimized by the addition of N fertilizer throughout the season. The high concentration of pre-existing soil inorganic N could be the reason why the N fertilizer treatments did not consistently outperform the unfertilized control.

There were no yield effects on carrot when foliar seaweed (marketed to contain cytokinin) was applied. The plants responded, instead, to N fertilizer source. Hemphill (1981) studied the effect of a cytokinin containing algae-based product on tomatoes, potatoes, carrots, celery, and strawberries. Hemphill (1981) found that only tomatoes produced greater yield in response to cytokinin, because the plant produced more fruits. This experiment agrees with Hemphill's findings for carrots. While Hemphill (1981) states that other researchers have seen a positive response in carrots, it may be that phytohormone-containing products are more effective on fruiting plants, and have less impact on root crops.

There were other significant differences among fertilizer treatments in 2014. Mg content of the carrot leaves was higher in all N fertilizers compared to the control (Figure 5). Mg plays an important role in plants as the center of the chlorophyll pigment. In a future study, the leaf greenness should be measured with a chlorophyll meter or the leaf tissues should be analyzed for chlorophyll to determine if there is a correlation between the higher Mg level in the treated leaves and the greenness of the leaves. Higher Mg levels in leaf tissue may be of interest in the context of fortified food. Vegetables with higher nutrient contents may be more desirable in the vegetable market.

The cyano-fertilizer treatment produced carrots with a greater average length than the unfertilized control in 2014 (Table 9). There was a significant interaction between N treatment and foliar seaweed in average carrot diameter (Table 7). The control + foliar seaweed treatment produced carrots with a smaller diameter (4.9 cm) than the non-hydrolyzed fish + foliar seaweed (5.5 cm) and the cyano-fertilizer + foliar seaweed (5.5 cm). The cyano-fertilizer + foliar seaweed (5.5 cm) produced larger carrots than both the hydrolyzed and non-hydrolyzed fish fertilizers with no foliar seaweed (5.1 cm) ($p<0.05$). While increasing yield is the ultimate goal of most farmers, carrot diameter may be a parameter that is best reduced. The USDA grading guideline specifies that the maximum diameter of a “U.S. No 1” carrot should not exceed 3.8 cm. All of the 2014 carrots were wider, putting them in the “U.S. No. 2” category, with a diameter maximum of 7.6 cm (USDA AMS, 2016).

In 2014, there were several differences in stress and root deformations as a result of treatments. Averaging across seaweed treatments, the hydrolyzed fish fertilizer reduced root knobs compared to the non-hydrolyzed fish fertilizer (Figure 6). There was a more pronounced difference in root knobs among treatments when no liquid seaweed was applied, and non-hydrolyzed fish fertilizer with no seaweed had a higher incidence of knobs compared to cyano-fertilizer and the hydrolyzed fish fertilizer treatments with no seaweed. Within the non-hydrolyzed fish fertilizer treatment, the addition of foliar seaweed reduced root knobs (Figure 6). For root knobs, F-test statistics show that the foliar seaweed had a greater influence over the incidence of the deformity than the N treatments (Table 7).

Cyano-fertilizer and hydrolyzed fish fertilizer also reduced the percent of carrots that did not fully develop in length (Figure 7) compared to the control. Within the unfertilized control, the addition of foliar seaweed decreased the incidence of under-developed roots (Figure 7).

These results complement the root knob findings. The N fertilizers and the foliar seaweed had equal levels of influence over root underdevelopment.

Because the N fertilizers all produced carrots with similar incidence of root knobs to the control, it is difficult to attribute the difference between hydrolyzed and non-hydrolyzed fish fertilizers to the N source. As there was only one case in which the addition of foliar seaweed reduced root knobs within a treatment, it is also difficult to conclude that the foliar seaweed treatments will improve carrot root quality by reducing common deformities. In the case of underdeveloped roots, however, the control had the most frequent occurrence of underdeveloped roots, and two of the N fertilizers were significantly lower. There were no significant differences in underdeveloped roots with or without foliar seaweed within N fertilizer treatments. These findings suggest that an unknown characteristic of the N sources may be responsible for reducing underdeveloped roots. Interestingly, within the control, the addition of foliar seaweed decreased underdeveloped roots. It is possible that the N fertilizers and the foliar seaweed may share the characteristic responsible for decreasing underdeveloped roots, such as potassium supplementation. Hamza and Suggars (2001) found that exogenously applied phytohormones improved the quality of turf grass only when used in conjunction with N fertilizers, but the combination led to higher quality grasses than N fertilizer alone. In terms of the marketability and quality of the carrots produced in this study, the results mostly agree with Hamza and Suggars' (2001) results, with the exception of the control and underdeveloped roots.

The average stress index of each treatment is listed in Table 10. Stress was generally very low in this study, and most plants grew with no stress symptoms. Stress was of interest due to manufacturers' claims that foliar seaweed applications can reduce plant stress. This claim was difficult to properly examine in the absence of general stress symptoms in the plots. Rows treated with cyano-fertilizer earned an average of 0.13 stress points fewer than hydrolyzed fish fertilizer rows which received 1.3 points on average (p=0.03). The liquid seaweed treatment averaged across N fertilizers nearly showed a significant decrease in stress compared to no foliar seaweed by an average of 0.7 points (p=0.06). There was a significant interaction between N fertilizer and foliar seaweed. Fish fertilizers, with the addition of foliar seaweed, increased stress compared to other treatment combinations. These results suggest that in the absence of fish fertilizer, foliar seaweed may have reduced stress. However, the combination of fish fertilizers and foliar seaweed increased, or did not decrease, plant stress. The reason for this interaction is unclear; a possible explanation may be that with a combination of products originating from the oceans, plant-toxic levels of micronutrients may have caused some of the stress observed in this experiment. In a future study, the heavy metal and micronutrient contents of all fertilizers should be compared and investigated for possible detrimental effects of additive micronutrient levels.

Not surprisingly, all of the N fertilizers left the soil with increased NO_3^--N concentrations compared to the control, but were comparable to each other (Table 11). All treatments left similar levels of post-season soil NH_4^+-N.

Although results varied between years, and few differences were observed in the variables measured, this study makes a strong argument that adding mid-season N from cyano-fertilizer or a fish fertilizer may benefit carrot growth and marketability characteristics. In 2014, soil N was increased, as expected, equally among N fertilizers compared to the unfertilized control.

The cyano-fertilizer performed at least as well as the fish fertilizers. The cyano-fertilizer produced a higher yield than the control in 2014, and produced a greater yield than the hydrolyzed fish fertilizer in 2015. While soil inorganic N levels were already considered adequate at the start of the experiment, yield improvement over the control and a commonly-used organic fertilizer provide promise for the effectiveness of cyano-fertilizer.

Foliar seaweed had interesting effects. The addition of a foliar seaweed treatment reduced the number of underdeveloped roots in the control and nearly showed a significant decrease in stress across the control and cyano-fertilizer treatment, but when foliar seaweed was applied in addition to fish fertilizers, stress was significantly increased. Because none of the results were consistent across years, it is difficult to draw conclusions about the usefulness of foliar seaweed applications to carrots.

CUCUMBERS

The ANOVA test results for the cucumber studies in 2014 and 2015 are listed in Table 12 and Table 13, respectively. F test statistics are only shown for significant results. All other variables mentioned in the measurements section above, which are not listed in Table 12, were statistically similar among N fertilizer treatments and between foliar seaweed treatments.

There were very few differences in the measured variables in this study. Except for the second harvest in 2015, all cucumbers had similar lengths and diameters at the time of harvest. The cucumber vines were similar in their plant stress indices, wet weights, biomass, and water contents. There was little to no insect pressure during this study, which was a surprising result, as controlling pests in organic cucurbits is often difficult.

In 2014, there was only one cucumber harvest due to an early frost. Non-hydrolyzed fish fertilizer treatment produced a greater number of cucumbers harvested compared to cyano-fertilizer (Table 14). Additionally, the non-hydrolyzed and hydrolyzed fish fertilizers produced higher yield than the cyano-fertilizer. In this season, the cyano-fertilizer produced 50% less yield than the control.

In 2014, the hydrolyzed fish fertilizer left the greatest residual soil NO_3^--N compared to the other treatments and the control (Figure 8).

In 2015, the season was long enough to support two cucumber harvests, and the treatment results were almost the opposite of the 2014 results. During the first harvest, all of the N fertilizers produced similar yields and numbers of fruit harvested (Table 14). During the second 2015 harvest, the non-hydrolyzed fish fertilizer produced fewer cucumbers than the cyano-fertilizer and the control, and the hydrolyzed and non-hydrolyzed fish fertilizers produced less yield compared to the cyano-fertilizer and the control. The cyano-fertilizer may have performed similarly to the fish fertilizers during this second harvest, but cyano-fertilizer application was limited by a predatory microbe, making N application rate similar to the control later in the season. One possible explanation for decreased yield may be that the additional N from fish fertilizers encouraged more vegetative growth in the cucumber plants, whereas the lack of additional N from the control and cyano-fertilizer encouraged fruit set.

The cucumbers in this study performed well, even in the unfertilized control. No plants showed signs of N or other nutrient deficiency. While the cucumbers did respond to N fertilizers in both years, yield impacts can be described as limited; these findings are in agreement with those observed by Van Eerd et al. (1999).

Foliar seaweed did have a significant impact on cucumber growth measured at the second harvest in 2015. Those cucumber plants receiving foliar liquid seaweed produced cucumbers with a wider average diameter (6.23 cm) than those without (5.96 cm) ($p=0.03$). Non-hydrolyzed fish fertilizer produced shorter cucumbers (22.2 cm) than the control + foliar seaweed treatment (23.8 cm) ($p<0.05$). While increasing yield is the ultimate goal of many farmers, increasing cucumber diameter may not be beneficial to the farmer. According to the USDA AMS grading guidelines, “U.S. Fancy” and “U.S. Extra No. 1” cucumbers have a diameter maximum of 6.0 cm (USDA AMS, 2016). These grades are a higher priced product than the “U.S. No. 1 Large” Cucumbers which have no diameter limit. In this experiment, the cucumbers receiving foliar seaweed applications would be classified as “U.S. No. 1 Large” while the untreated cucumbers would fall into the “U.S. Extra No. 1” category based on diameter. This may also be an indication that the foliar seaweed influenced maturation rate. If the cucumbers matured faster, and were harvested sooner, they may have fallen into the “U.S. Extra No. 1” category at an earlier date. This result would be of interest to a farmer trying to complete as many cucumber harvests as possible in one season and aiming to sell cucumbers in the highest priced “U.S. Extra No. 1” category.

There were no significant differences in post-season soil inorganic N in 2015. In 2014, the hydrolyzed fish fertilizer treatment had a greater level of residual soil NO_3^--N than the control and the other treatments. This may be due to the hydrolyzed nature of the fertilizer, attractive for its immediately plant available N. The cyano-fertilizer and the non-hydrolyzed fish fertilizer may need a longer period of time for the organic N to mineralize. This could be supported by the 2015 findings, in which no soil N differences were observed at the end of a longer season.

CONCLUSIONS

When choosing an organic vegetable variety for a future study, using an heirloom variety instead of a hybrid designed for stability may present more measurable differences between treatments. The pre-existing soil chemistry in the field at the CSU Horticultural Research Farm may have been such that macro- and micro-nutrient deficiencies were not observed, and overall stress levels were quite low. Most of these results were not consistent or pronounced enough to prove the benefit of applying any particular combination of treatments to carrots or cucumbers, but would warrant further exploration into the potential impacts of cyano-fertilizer and foliar seaweed on overall quality and yield of vegetables, as higher rates or lower pre-season soil fertility may be more revealing. In the variables which were impacted by treatment, the cyano-fertilizer performed similarly to the other fertilizers except in the 2014 cucumber harvest. Therefore, farm-grown cyano-fertilizer may be a viable option for organic farmers to reduce the environmental impact of their operation, but more yield analysis is required.

In this study, the application of products containing phytohormones seemed to have little impact, except for producing slightly wider and longer cucumbers of the seaweed treated cucumbers in the second harvest of 2015, and decreasing carrot root knobs. Although no phytohormones were found in the 2015 foliar seaweed treatment in the panel conducted by the Colorado State University Proteomics and Metabolmics Facility, there are many more phytohormones than the panel detected. If there would be an effect of applying foliar seaweed, it would be most expected in a fruiting plant, impacting the fruits. Other researchers, such as Hemphill (1981), have seen a positive response from fruiting plants, but find less response from root crops. Hemphill (1981) found that out of tomato, strawberries, celery, carrots, and potatoes, tomato was the only plant to show a positive response. In that study, a lack of response in strawberries was theorized to be the result of a heavy rain shortly after foliar application. Future studies on the impacts of phytohormones in organic fertilizers on fruiting plants should test higher seaweed application rates as well as perform more comprehensive phytohormone assays for treatments and plant materials.

CHAPTER 2

EFFECT OF LIQUID ORGANIC FERTILIZERS AND SEAWEED EXTRACT ON BELL PEPPER (*Capsicum annuum*) GROWTH CHARACTERISTICS UNDER GREENHOUSE CONDITIONS

SUMMARY

Bell peppers (*Capsicum annuum*) were grown in a greenhouse experiment in 2015 at the Colorado State University Plant Growth Facility to identify fertilizer and foliar seaweed application effects on yield, stress, and growth characteristics of bell peppers under N deficient conditions. Cyano-fertilizer was produced in a 75 L tank and evaluated in this study to compare effects of farm-grown fertilizer and commonly-used organic fertilizers. Hydrolyzed and non-hydrolyzed fish fertilizer, and cyano-fertilizer treatments were applied at 30 kg N ha^{-1} in split applications approximately every 7 days over a 135 day growing period. Control plants received no supplemental N. Each treatment, including the control, was repeated with the addition of Neptune's Harvest foliar seaweed, and again with PGR foliar seaweed for a total of three seaweed treatment combinations for each N fertilizer treatment. Foliar seaweed treatments were applied at the manufacturer's recommended dilution rate, and both brands were applied to evenly coat all pepper leaves. The leaves of the pepper plants contained similar concentrations of P, K, B, Fe, and Cu. Non-hydrolyzed fish treated plants had a greater concentration of leaf N than the control. The plants receiving cyano-fertilizer contained more leaf S than the hydrolyzed fish fertilizer and the control. The leaves of the control plants contained significantly higher concentrations of Ca, Mg, Mn, Mo, and Zn than some of the other treatments. All treatments produced pepper plants with similar leaf to stem ratios by weight, leaf and stem wet weights, leaf

and stem dry weights, leaf numbers, and water contents. The leaves from plants treated with non-hydrolyzed fish fertilizer contained higher levels of abscisic acid 12-oxo-phytodienoic acid (OPDA) than the control. There were no significant differences in fruit β–carotene levels among treatments. Plants treated with cyano-fertilizer and non-hydrolyzed fish fertilizer produced 12.2% and 13.3% more yield than the control group. Foliar seaweed applications averaged across all treatments produced pepper plants with differing branching height (distance to first branch) to total plant height ratios. Foliar seaweed treatments also presented significant differences in the total number of branches on each plant. The N fertilizers also impacted the total number of branches, as the control plants had significantly fewer branches than all other N treatments. The non-hydrolyzed fish fertilizer and cyano-fertilizer increased the number of dead or abscised flowers starting on day 109. At harvest, the control group had the greatest number of dead flowers and leaf abscissions. Plant stress was monitored using an original point-based scale ranging from 0-5. Control plants were more stressed than N fertilized plants at harvest, and the foliar seaweed treatments had no impact on stress. Foliar seaweed applications impacted pepper color and shape.

INTRODUCTION

Bell peppers (*Capsicum annuum*) are a warm-season annual (although they can be perennial in warm climates) member of the Solanaceae family. *Capsicum annuum* originated in Central and South America and the Caribbean. There are numerous varieties within the species, of which bell peppers are one. Bell peppers are green when immature, and turn a variety of colors as they ripen depending on plant variety (USDA NRCS, 2016).

Organically-grown greenhouse bell peppers can be grown year-round and fetch a higher price for producers than conventionally grown bell peppers (USDA AMS, 2016). Additionally, organically-grown peppers may provide consumers with greater health benefits than conventionally grown bell peppers due to greater levels of antioxidants (Hallmann and Rembialkowska, 2012).

Increasing yield in an organic system could be of great benefit to the producer. The most common method for increased yield in agriculture is to provide plants with supplementary N. There have been many field studies conducted on N supplementation of peppers, and in most experiments, supplying N to pepper plants increased the number of flowers and the fruit yield (Maynard et al., 1969). Bar Tal et al. (2001) studied the optimum N rate for greenhouse grown peppers in an aeroponic system. The optimum N concentrations for maximum stem and leaf dry matter production were in the range of 56 to 64 mg L^{-1}. Due to the high intrinsic N in the soil at the field sites where fertilizers were previously tested (as reported in chapter 1), this study was designed to be N deficient in the control treatment. Urrea-Lopez et al. (2014) designed an N deficient fertilizer solution for bell peppers grown on perlite by delivering a 35 mg L^{-1} solution of N twice weekly. However, this level of N was not low enough to significantly reduce pepper yields. The average N content of cyano-fertilizer in this study was expected to be 23.3 mg L^{-1}, delivered weekly, with an initial inorganic soil N level of 10 kg ha^{-1}. Because the cyano-fertilizer is slightly less concentrated in N compared to the solution used by Urrea-Lopez et al. (2014), it was expected that the pepper plants would be deficient enough to reduce pepper yield, where the 35 mg L^{-1} N solution did not. Using a lower N application rate in this study was also an attempt to magnify the potential impacts of the foliar seaweed applications on flowering and fruit set, as

Maynard et al. (1969) found that supplemental N fertilizer often increased flowering and fruit yield.

Another potential method to increase yield is to impact growth characteristics by applying exogenous phytohormones. It is well established that auxin and cytokinin have an impact on plant growth characteristics such as cell elongation and cell division. Few studies have observed impacts of applied phytohormones on bell peppers. Tiwari et al. (2012) found that auxin impacts fruit set in peppers, and gibberellin may play an important role in preventing flower and fruit abscission. Interactions between hormones are important as well; for example, Tiwari at al. (2012) found that auxin and gibberellin applied together seem to promote a balance between cell elongation and expansion during fruit growth.

Jasmonic acid (JA) is a signaling hormone that is related to plant response to biotic and abiotic stress. The addition of JA to crops is being explored as a method to inexpensively increase resistance to pests that would otherwise significantly reduce yield (Thaler, 1999). Bosch et al. (2014) found that JA was required to signal a stress response in tomatoes, but that a JA precursor, 12-oxophytodienoic acid (OPDA), could substitute for JA as OPDA can readily be converted to JA in the plant. Taki et al. (2005) attempted to understand if OPDA served a specific role without being converted to JA. These researchers found that several genes in *Arabidopsis* responded only to OPDA, and not to JA. The signaling pathway also differed, showing that OPDA can readily be converted to JA if the plant requires it, but that OPDA acts independently in response to physical wounding in the plant.

Fruit content of some secondary plant compounds, such as β-carotene, are of great importance to consumers due to health benefits of eating fortified food. In a 2012 study by

Hallmann and Rembialkowska (2012), organically grown bell peppers contained more β-carotene and other beneficial secondary compounds compared to conventionally grown bell peppers. While it is known that β-carotene acts as an antioxidant, it is also being explored as an anti-allergen and an anti-cancer agent (Teng et al., 2016; Hiragun et al., 2016).

The aim of this study was to investigate the effects of organic liquid N fertilizers and foliar applications of liquid seaweed on plant growth characteristics such as branching, flowering, and leaf and fruit abscission, and their contributions to overall yield and quality of a bell pepper crop compared to an N deficient control.

MATERIALS AND METHODS

STUDY SITE

This study was carried out at the Plant Growth Facility on the CSU campus from spring to summer of 2015. The greenhouse allowed natural light in, and provided supplemental light to extend short days or rainy days to 12 hours.

PEPPER VARIETY

The bell pepper variety "Aristotle" was chosen for this study. The Aristotle pepper is described as a 73 day, widely adaptable variety that does well all over the United States. It is a green to red pepper with a "blocky" shape. The vegetative part of the plant can be 50 to 61 cm in height. The variety is described as having intermediate resistance to common diseases, and is free of anthocyanins, which causes purpling during stress. Aristotle pepper seeds were purchased from Harris Seeds in Rochester, NY.

EXPERIMENTAL DESIGN

The pots were arranged in a randomized complete block design. The treatments were handled as a 4x3 factorial, with four soil N treatments (control, hydrolyzed fish fertilizer, non-hydrolyzed fish fertilizer, and cyano-fertilizer) each receiving three foliar treatments (none, PGR seaweed extract, and Neptune's Harvest seaweed extract). The control plants received no supplemental N, and the "no foliar" plants received no foliar seaweed applications. Three replications of each treatment combination were prepared, for a total of 36 pots. The pots used were black, 7.6 L plastic pots with 5 drainage holes on the bottom. Each pot was approximately 21.5 cm tall, with a 22.8 cm diameter. To keep the soil media in the pot, a 25 by 25 cm piece of fiberglass screen was fitted to the bottom of the inside of the pot. Each pot was placed in a black seeding tray which was tilted towards the pot to capture water and fertilizer applications that percolated through the pot, making that liquid available for the soil to re-absorb. From transplant to harvest, the growing season was extended to 135 days in the greenhouse in an attempt to acquire multiple pepper harvests.

SOIL MIX

This study was designed in response to the high pre-plant inorganic N and organic matter levels present in the field study plots. The productive soils may have been contributing more N to the test plots than would be ideal for a N fertilizer comparison. A preliminary study was carried out to optimize the plant growth media, while minimizing intrinsic N. Three growing media were mixed to minimize existing nutrients while maximizing water holding capacity: sand, perlite, and vermiculite. The three components were mixed in 6 different ratios by volume to determine the mixture with the greatest water holding capacity, based on the lowest % water loss after 3 days. The summary of the tests is in Table 15.

The mixture of 60% sand and 20% each of vermiculite and perlite was chosen because it held the second most volume of water, but lost the second least amount of water weight in three days, showing it was the most well rounded mixture.

The wetted soil mix was sampled and sent to the Colorado State University Soil, Water, and Plant Testing Laboratory following an extraction with 2M KCl. Analysis was performed with a Lachat auto-analyzer. There was some inherent N in the wetted soil mix; it contained on average 3.5 mg kg^{-1} NH_4^+-N and 0.40 mg kg^{-1} NO_3^--N. This was presumably contributed from either the tap water or contamination of the soil constituents.

SEEDING AND TRANSPLANTING

On February 25th, 2015, a 72 cell Rockwool starter plug tray was watered with a vinegar solution of pH 6.5 to provide a favorable pH for seed germination. On February 6th, 2015, 150 Aristotle pepper seeds were planted into the Rockwool, watered daily, and warmed with a heating pad below the tray. The seedlings emerged on February 13th, 2015. On February 25th, 2015, the peppers were thinned and transplanted into the pots for the remainder of the experiment.

TREATMENTS AND FERTILIZATION

Bell peppers were grown in a low nutrient soil media in order to control available N more carefully. For this reason, nutrients besides N had to be supplemented. 100 L of N-free Hoagland's solution in powder form was purchased from Bio-World (Dublin, OH). The product contains macronutrients and micronutrients necessary for plants. The manufacturers recommend adding N, sucrose, cytokinin, and auxins as needed. This solution provided the appropriate background nutrient supply to manipulate N treatments and foliar seaweed applications as the experiment required. Product instructions call to dissolve 1.9 g L^{-1} to provide essential nutrients for hydroponic N-fixing plants and plants grown on vermiculite. During fertilization, half-strength (0.95 g L^{-1}) N-free Hoagland's solution was mixed together with the N fertilizers.

The target total N rate for peppers was 40 kg N ha^{-1} so that the minimum N needs of the plants would be met, in contrast with the field studies where the pre-existing N condition of the soil may have provided all necessary N. The target was reached by all fertilizers. The three N fertilizers used in this experiment were: cyano-fertilizer, hydrolyzed fish fertilizer, and non-hydrolyzed fish fertilizer. The cyano-fertilizer was grown in a 75.7 L fish tank in the greenhouse using a scaled-down version of methods described by Barminski et al. (2016). Neptune's Harvest hydrolyzed fish fertilizer and Alaska non-hydrolyzed fish fertilizer were purchased from Neptune's Harvest (Gloucester, MA) and Fort Collins Nursery (Fort Collins, CO), respectively. The three fertilizers varied in N concentration and were applied at equal N rates (Table 16). N application rates were gradually increased throughout the season due to the low nutrient holding capacity of the soil mixture and the increasing nutrient demand of the growing plants.

The N concentration of the cyano-fertilizer was measured the day of fertigation using a Hach DR3900 Benchtop Spectrophotometer (Loveland, CO) to measure Total Kjeldahl Nitrogen. For each treatment, the fertilizer and half-strength Hoagland's solution powder were mixed thoroughly in a pitcher. The fish fertilizers were mixed to match the N concentration of the cyano-fertilizer by diluting to volume with water. Cyano-fertilizer was added full strength to the pitcher and mixed with the half-strength Hoagland's solution powder. Fertilizer was allocated from the mixing pitcher into each pot using a graduated cylinder. Each pot received the same volume of water during fertilizations. The dates and N application rates are listed in Table 16.

PGR Organic seaweed extract was purchased from Johnny's Selected Seeds (Winslow, ME). Neptune's Harvest organic seaweed extract was purchased from Neptune's Harvest (Gloucester, MA). The seaweed was applied foliarly using a 900 mL spray bottle. The seaweed extracts were mixed according to the manufacturers' recommendations. 1.1 mL of PGR seaweed extract was diluted into 825 mL of water, and 3.2 mL of Neptune's Harvest seaweed extract was diluted into 825 mL of water, and equal volumes were applied to the leaves of the assigned pepper plants. The average volume per spray from the bottle was found to be 1 mL. Similar to the N fertilizer rate, the seaweed application rate was increased through the season following manufacturers' recommendations to cover each leaf adequately. The dates and volumes of liquid seaweed applied to the foliage of each plant are listed in Table 17.

On April 15th, 2015, 25 cm x 25 cm squares of aluminum foil were folded over the exposed soil to eliminate soil surface algal growth. April 16th, 2016, the pepper plants were treated with Entrust® SC Naturalyte®, a fermented spinosad product, to control thrips.

IRRIGATION

Each pot received 1 L of water, every other day starting on February 25th, 2015. On the days that fertilization occurred (Table 16), the water supplied in the fertilizer mix was the only water applied in that day. The total irrigation supplied to the peppers over the growing season was 57.4 L pot^{-1}.

MEASUREMENTS

Plant height, leaf number, plant stress, and flower number measurements were taken each week. Plant height was measured to the tip of the tallest leaf. Plant stress was quantified using an original 5 point scale that was created to encompass all symptoms such as nutrient deficiency, pest stress, diseases and more. On this scale, 0 stress points represents a wholly healthy plant, and a score of 5 represents a plant that is dead or beyond the permanent wilting point. Flowers were separated into five categories: green flowers, dead flowers or abscissions, white flowers, finished flowers, and peppers (Figure 9).

On July 10th, 2015, the peppers were harvested from the plants. Each pepper was measured, weighed, and photographed. The peppers had developed noticeably different shapes. Categories were created to classify the shapes: traditional blocky, bell-shaped peppers (bell), elongated peppers (long), and peppers that curved dramatically (curved). The dominating color of each fruit was also recorded. Examples of the shape and color differences can be seen in Figure 10. Once physical measurements were taken, the peppers were sent to the Proteomics and Metabolomics Facility at Colorado State University and were analyzed for β-carotene concentration utilizing LC-MS.

On July 11th, 2015, the pepper plants were cut 1 cm from the soil surface and removed from the pots. Each plant was laid on a 1 cm grid and photographed. The stem and the branches were carefully measured to determine the number and location of branches, as well as the location of every leaf and flower. Additionally, distances between branches were measured, and locations where leaves, flowers, and fruits were lost were noted. The average number of branches, the distance between branches, the number of abscissions that had occurred, and the final leaf and flower counts were recorded. Additionally, the ratio of the height of the first branch to the total height of the plant was calculated. An example of the branch mapping measurements can be seen in Figure 11. The plants were then weighed, separated into leaves and stems, and re-weighed. Several leaves from each plant were sent to the CSU Proteomics and Metabolomics Facility. Utilizing UPLC-MS, phytohormones were extracted and measured. The assay is designed to measure cytokinins, auxins, gibberellins, brassinosteroids, jasmonates, salicylates, and more. The remainder of the plant material was dried at 49^{o}C for 5 days. The dried leaves and stems were re-weighed to obtain dry matter content of each. Leaf tissue was ground to a fine powder and sent to Ward Laboratories Inc. in Kearney, NE to be analyzed for N, P, K, S, Ca, Mg, Zn, Fe, Mn, Cu, B, and Mo using ICP spectroscopy following an acid digestion. Each pot was soil sampled on July 11th, and samples were extracted with a 1:10 ratio of 2M KCl. The extracts were analyzed for NO_3^--N and NH_4^+-N by automated colorimetry at the CSU Soil, Water, and Plant Testing Laboratory in Fort Collins, CO.

STATISTICAL ANALYSIS

All statistics were performed using Statistical Analysis System 9.4 (Cary, NC). The procmixed statement was used, and the experimental design was run as a 4x2 factorial. Treatment and foliar seaweed applications were treated as fixed effects. and Block (or replicate) was treated as a random effect. The slice (F-test) statement was used to analyze effects of foliar seaweed extract. An adjusted F-test of fixed effects was performed using the REML method. Least square means were estimated with the lsmeans statement. Least square means were compared with the pdiff statement. P-values <0.05 were considered significant in all cases.

RESULTS AND DISCUSSION

Results from the ANOVA test are shown in Table 18. Only significant results are listed. All other measured variables produced plants or fruits with statistically similar values.

Nutrient concentrations in the dried pepper leaves can be seen in Table 19. There were several noteworthy differences in leaf nutrient concentration. The leaves of the pepper plants contained similar levels of P, K, and Cu. Plants treated with non-hydrolyzed fish fertilizer contained more N in their leaves than the control. The interaction between N fertilizer and foliar seaweed was significant ($p<0.10$), but the results do not follow a clear pattern (Table 18). An example can be seen within the cyano-fertilizer treatment; plants treated with Neptune's seaweed had lower leaf tissue N than those treated with PGR seaweed ($p<0.05$). This result may have been induced by the increased salicylic acid concentrations found in the cyano-fertilizer, inducing stress in pepper plants, but the cytokinin content of the PGR seaweed may have counteracted this effect (Table 2). This pattern was not repeated elsewhere within the N fertilizer treatments.

The plants receiving cyano-fertilizer contained more S than the hydrolyzed fish fertilizer and the control. As an essential element for the construction of amino acids and a component of vitamins which are beneficial to humans, such as vitamin A, increased leaf tissue S analysis may be of interest in the context of fortified foods. In a future study, the S content of the N fertilizers and foliar seaweed treatments should be measured and compared in an attempt to understand the reason for the increased leaf tissue S in the cyano-fertilizer leaves. Sulfates are provided to feed the cyanobacteria in the cyano-fertilizer, and organic or excess inorganic S may be carried over during application to the plant. Analysis is necessary, however, to determine forms and quantity of S in the fertilizers.

The leaves of the control plants contained significantly higher levels of Fe, Ca, Mg, Mn, Mo, and Zn than some of the other treatments (Table 19). According to American Agricultural Laboratory (2013), all treatments produced pepper plants that were N deficient. However, the non-hydrolyzed fish fertilizer produced plants with leaves containing a greater concentration of N compared to the control. Although the non-hydrolyzed fish did not produce statistically larger plants, the increased leaf tissue N may be the reason that the non-hydrolyzed fish fertilizer contained lower levels of immobile micronutrients than the control plants. All treatments produced pepper plants with similar leaf to stem ratios by weight, leaf and stem wet weights, leaf and stem dry weights, and water contents.

The leaves from the non-hydrolyzed fish fertilizer treated pepper plants contained more than six times higher levels of abscisic acid than the control (Table 20); the leaves from all other treatments were not different from the control or the non-hydrolyzed fish fertilizer. The non-hydrolyzed fish fertilizer leaves also contained a greater concentration of 12-oxo-phytodienoic acid (OPDA), a wound-stress signal and a cyclopentenone precursor of jasmonic acid (JA). However, JA was not different among treatments. Many phytohormones that were expected to be detected were not found in this assay, including cytokinins, auxins, phaseic acids, benzoic acid, salicylic acids, brassinosteroids, and gibberellins. There were no significant differences in β–carotene concentrations in the fruit among treatments.

Plants treated with cyano-fertilizer and non-hydrolyzed fish fertilizer produced 12.2% and 13.3% more yield (g) than the control plants, respectively (Figure 12). All other treatment comparisons were not different from each other. Overall, the pepper plants were smaller and more chlorotic than what would be expected under a high N scenario. These results contrast with the yield results from Urrea-Lopez et al. (2014), in which pepper yield was not significantly decreased by N deficient fertilizer treatments.

The foliar seaweed treatments had an impact on pepper fruit color and shape (Figure 10). The PGR foliar seaweed treatment increased the number of green peppers harvested (Figure 13). All other treatments had similar numbers of green and red fruits. As seen in Figure 14, the foliar seaweed treatments reduced the number of "bell" shaped peppers compared to the control, and they increased the number of "long" shaped peppers compared to the control. All treatments had similar numbers of "curved" shaped peppers. As found by Tiwari et al. (2012), the interplay of phytohormones can influence fruit set. It may be that in the case of the "long" shaped peppers, the cell elongation rate exceeded the cell division rate. This may be explained by unbalanced levels of auxin and cytokinin. If the auxins and cytokinins in the PGR seaweed (none found in Neptune's Harvest, see Table 2) disrupted the balance of these two important phytohormones, the effect may be the opposite of the intent of the product. This theory, however, does not explain the occurrence of "curved" peppers, unless they are the result of a different process, such as improper flower development.

While there were no significant differences in the height to the first branch, meaning that branching started at approximately the same height in all treatments, Neptune's Harvest foliar seaweed, averaged across N treatments, produced pepper plants with differing branching height (distance containing branches, or distance above the first branch) to total height ratios (Figure 15). The Neptune's Harvest seaweed produced plants with shorter branching sections compared to the PGR seaweed and the control. These plants were described as "tree-like" as they had more stem height than branches, whereas the control and PGR seaweed treatment produced plants with more proportionate branch and stem sections. Foliar seaweed treatments also presented significant differences in the total number of branches on each plant (Figure 16). PGR seaweed produced plants with a greater number of branches than no seaweed at all, and the Neptune's Harvest seaweed was statistically similar to the control and the PGR seaweed treatments. The N fertilizers also impacted the total number of branches, as the control plants had significantly fewer branches than all other N treatments which were comparable in total branch number (Figure 17). Plant height was statistically similar on all measurement days.

The N fertilizer treatments produced differing numbers of living flowers on several measurement days. In particular the non-hydrolyzed fish fertilizer had the greatest number of dead or abscised flowers on day 87 (Table 21). On day 126, the non-hydrolyzed fish fertilizer increased the number of dead flowers, while the living flower numbers were unaffected by treatment. Foliar seaweed did not impact flowers on most measurement days or at harvest, but on day 87 PGR seaweed increased flower death (2.3) compared to Neptune's Harvest seaweed (0.67) and the control (0.50) ($p<0.05$). On day 97, PGR seaweed increased flower death (3.2) compared to the control (1.5). All leaf abscissions and dead flowers were summed on the day that the pepper plants were harvested for a total percentage of abscissions and abortions compared to the percentage of leaves and flowers that remained vital at the end of the experiment. The percentage of abscissions and dead flowers compared to the total number of leaves and flowers was deemed "abscission events." In this metric, hydrolyzed fish fertilizer presented with a 13.5% greater number of abscission events than the control (Table 21). These results do not present a clear pattern of leaf loss and flower death that would lead to a conclusion about product application for increased flower vitality. The treatments with the most abscissions changed nearly weekly after day 87. At the end of the experiment, however, the control plants had the fewest living leaves and flowers, suggesting that the N provided in the experiment was not sufficient to continue to support the leaves that the young plant grew. Additionally, PGR seaweed may not be the best choice in foliar seaweed products for bell peppers. Neptune's Harvest seaweed and the control did not impact leaves and flowers, but the PGR seaweed increased flower abortions for 2 consecutive measurement days. Due to the mixed nature of these products, it is difficult to determine if the phytohormone content of these products was influencing the flowers. Based on the abscisic acid content in the leaves (see Table 20), it would be expected that the hydrolyzed fish fertilizer would have the greatest number of abortions and abscissions upon harvest. This was not the case, as the hydrolyzed fish fertilizer had the smallest percentage of abscissions on harvest day. The absence of gibberellins in the leaf tissue is interesting in the context of flower and leaf abscission. Tiwari et al. (2011) found that gibberellins play a role in preventing flower abortion. In a future study, applying gibberellins to the plants and observing changes in flowering behavior would increase understanding of this dynamic. The greater number of abscissions seen in the control plants may have led to the higher concentrations of immobile nutrients (Fe, Ca, Mg, Mn, Mo, and Zn) seen in the leaf tissue analysis (see Table 19). These nutrients may have remained in the plant as leaf tissue senesced towards the end of the experiment.

Significant differences in plant stress were observed on days 67, 97, and 145 (Table 22). The non-hydrolyzed fish fertilizer treated plants were the most stressed on day 67. On days 97 and 145, the control plants were more stressed than the fish fertilizers, and the cyano-fertilizer plants were not different from any of the treatments. Liquid seaweed treatments had no impact on stress. As products marketed to support plants through stress, the foliar seaweed treatments had surprisingly little impact on bell peppers in a low N environment.

There were also no significant differences in post-season soil NO_3^--N or NH_4^+-N concentrations, suggesting that the growing media may not have retained nutrients well, and that the roots scavenged all available N.

While there were several interesting impacts of the fertilizer treatments in this study, there were not many clear patterns indicating that one of the treatment combinations tested in this study was the most beneficial combination for greenhouse peppers. Yield was positively impacted by the addition of two of the three fertilizers, and branching characteristics were impacted by the addition of foliar seaweed. However, using foliar seaweed decreased the number of fruits that were “bell” shaped. As this is the marketable shape of bell pepper, it would seem that it may be advantageous to growers to not use foliar seaweed products on peppers. While PGR seaweed increased the total number of branches, all of the N fertilizers increased branches compared to the control, resulting in a similar impact. From this experiment, it is not clear that increasing branching would increase yield, but in an adequate N environment, that may be the case. Another important observation was that the PGR seaweed increased the number of green peppers compared to Neptune’s harvest seaweed and the control. This may be of interest to producers who are trying to deliver a consistent product of one color of either red or green peppers. These results would suggest that in a greenhouse environment, using a N fertilizer should be adequate, but the addition of foliar seaweed may impact the crop in very specific ways according to the grower’s needs, such as harvesting more green fruits. It is difficult, however, to determine the mechanism by which the Neptune’s Harvest foliar seaweed treatment manipulated plant growth characteristics, since no phytohormones were detected in the product. This unanswered question warrants exploration, as other traits of the product, such as micronutrient concentrations, could be influencing these plant growth characteristics.

Stress was decreased in this study with the addition of N fertilizers, and foliar seaweed had no impact on plant stress. The leaves of the plants treated with hydrolyzed fish fertilizer contained a far greater level of ABA, a stress and dormancy signaling phytohormone, than the control. The hydrolyzed fish fertilizer leaves also contained higher levels of OPDA, another stress hormone, than the cyano-fertilizer. These results are not complementary to the finding that upon harvest, the control plants displayed the most stress.

When growing fruiting crops in a greenhouse, flowering is of the utmost importance. In this study, the flowering effects were so varied that drawing conclusions about the best product among those tested in terms of increasing flower vitality was not possible. In a future study, the pepper plants should be provided with the same treatments at higher N rates. While the goal of this study was to observe changes in a low N environment, the lack of adequate N could have prevented more profound relationships between N source and branching. Additionally, providing more N to encourage plants to produce more fruit could be illuminating in terms of the interesting shape and color impacts observed in this study.

CHAPTER 3

OVERALL CONCLUSIONS AND FUTURE RESEARCH SUGGESTIONS

In all studies, the results were not consistent, especially not in the two year field studies, thus, conclusions cannot be drawn about the benefit of adding foliar seaweed to a N fertilizer regimen. While the addition of a N fertilizer usually increased yields and quality, no one fertilizer was predominately more effective than the others. With the exception of one cucumber harvest, the cyano-fertilizer performed as well as the control and the other N fertilizers. The foliar seaweed had very few significant impacts except in the N-deficient pepper study. This may be due to the fact that the N fertilizers contain many of the same compounds as the PGR seaweed. Alternatively, impacts of foliar seaweed applications may be so subtle, that in a highly-productive, adequate N scenario such as the field studies, the subtle differences are not distinguishable. Most importantly, each of the fertilizers and the foliar seaweed products are mixtures of many components. This makes attributing plant growth and yield characteristics to one specific component of the treatments very difficult. For example, the PGR foliar seaweed increased the number of green bell peppers harvested. The PGR seaweed contains phytohormones as well as micronutrients and some macronutrients which could be influencing pepper maturity. This complicated evaluation of impacts.

When comparing hydrolyzed to non-hydrolyzed fish fertilizer, the differences are small and inconsistent. The non-hydrolyzed fish fertilizer contained higher concentrations of auxin and salicylic acid (Table 2). This may be due to the fact that the non-hydrolyzed fish fertilizer is not enzymatically degraded like the hydrolyzed fish fertilizer. While exact methods are proprietary, the non-hydrolyzed fish fertilizer is commonly thought to be cooked down to increase concentration. Therefore, it is not only surprising that phytohormones survived in the fish, but also that the compounds survived processing in both fertilizers. The fertilizers performed very similarly in the carrot study, although the non-hydrolyzed fish fertilizer increased yield compared to the control in 2014. On the contrary, the non-hydrolyzed fish fertilizer increased root knobs compared to the hydrolyzed fish fertilizer in 2014 when no foliar seaweed was applied and when N fertilizers were averaged over foliar applications. It is difficult to attribute a true increase in marketable yield to the non-hydrolyzed fish fertilizer in carrots because of the increase in root knobs. Fewer carrots treated with non-hydrolyzed fish would have been sold at a market. Both fish fertilizers increased cucumber yield compared to cyano-fertilizer in 2014, but decreased yield compared to cyano-fertilizer and the control in 2015. The hydrolyzed fish fertilizer increased residual soil NO_3^--N in 2014 compared to the other fertilizers and the control. As the 2014 cucumber season was the shortest experiment, this may show that the N from the hydrolyzed fish fertilizer is indeed more immediately plant available than other organic N sources, as the manufacturers claim. In the pepper study, the leaves of the plants treated with the fish fertilizers contained similar concentrations of all nutrients and leaf phytohormones. Towards the end of the pepper study, the plants treated with hydrolyzed fish fertilizer had fewer dead flowers and fewer abscissions than the non-hydrolyzed fish fertilizer, but the plants otherwise showed similar stress levels. The plants treated with the two fish fertilizers were similar in all other variables measured. There were two cases when the hydrolyzed fish fertilizer was more beneficial for growth characteristics (fewer carrot knobs and fewer pepper flower deaths), but these slight and few differences do not present clear conclusions that hydrolyzed fish fertilizer is more beneficial to vegetable yield.

The most valuable future work would be to increase the dependability of the cyano-fertilizer. Research has shown that cyano-fertilizer results in similar yields as other organic fertilizers, and may even influence other plant growth characteristics via the introduction of phytohormones. The greatest challenge in this study was keeping the cultures growing successfully. Spirulina is grown successfully in many coastal locations. Perhaps the difference in UV radiation or the more dramatic changes in air temperature are hindering the process. Once the fertilizer can be grown reproducibly, there is nothing preventing it from becoming a viable option for many farmers all over the country.

Phytohormone contents and relationships in cyanobacteria and concentrated seaweed fertilizer warrant a closer look, in particular, on plant growth impacts. If there are truly more beneficial plant growth regulators to be found in cyano-fertilizer, this would make the on-farm source even more attractive and economically competitive among organic farmers who are using other products for those reasons.

Incorporating a radioactive tag or fluorescence into cyanobacteria fertilizer culture would shed light on multitudes of topics. This would provide more information as to the bacteria's behavior during the growth process as well as forms of molecules that are taken up by plants following cyano-fertilizer application.

Lastly, an experiment similar to the bell pepper study, where soil media N was low, should be repeated at a higher N rate. In this experiment, the lower N rate resulted in more significant differences compared to the field experiments, but if more N was applied, differences in yield may have been more apparent.

TABLES

Table 1. Description of fertilizers. Fertilizers were used in test plots and a greenhouse study in Fort Collins, CO in 2014 and 2015.

Treatment Name	**2014**
Control	None
Control+Seaweed	foliar SeacomPGR Organic Seaweed Concentrate****
Cyano-fertilizer	cyano-fertilizer*
Cyano-fertilizer+Seaweed	cyano-fertilizer* + foliar SeacomPGR Organic Seaweed Concentrate****
Hydrolyzed fish	Neptune's Harvest Organic Fish Fertilizer***
Hydrolyzed fish+Seaweed	Neptune's Harvest Organic Fish Fertilizer*** + foliar SeacomPGR Organic Seaweed Concentrate****
Non-hydrolyzed fish	Alaska Fish Fertilizer**
Non-hydrolyzed fish+Seaweed	Alaska Fish Fertilizer** + foliar SeacomPGR Organic Seaweed Concentrate****
	2015
Control	None
Control+Seaweed	Neptune's Harvest Organic Seaweed Plant Food*****
Cyano-fertilizer	cyano-fertilizer*
Cyano-fertilizer+Seaweed	cyano-fertilizer* + foliar Neptune's Harvest Organic Seaweed Plant Food*****
Hydrolyzed fish	Neptune's Harvest Organic Fish Fertilizer***
Hydrolyzed fish+Seaweed	Neptune's Harvest Organic Fish Fertilizer*** + foliar Neptune's Harvest Organic Seaweed Plant Food*****
Non-hydrolyzed fish	Alaska Fish Fertilizer**
Non-hydrolyzed fish+Seaweed	Alaska Fish Fertilizer** + Neptune's Harvest Organic Seaweed Plant Food*****

**Cyano-fertilizer - average Total Kjeldahl Nitrogen: 23.3 mg kg^{-1} or <1% by weight*

***Alaska Fish Fertilizer - fertilizer grade: 5-1-1*

****Neptune's Harvest Fish Fertilizer- fertilizer grade: 2-4-1*

*****SeacomPGR Organic Seaweed Concentrate - fertilizer grade: 0-4-4*

****** Neptune's Harvest Organic Seaweed Plant Food - fertilizer grade: 0-0-1*

Table 2. Fertilizer phytohormone concentrations. The following phytohormones were present in the fertilizers used in 2014 and 2015 field studies and a 2015 greenhouse study in Fort Collins, CO. The N rate used to calculate kg ha^{-1} was 68 kg N ha^{-1}.

	Phytohormones Detected					
	Auxin	**Salicylic Acid**	**Cytokinin**	**Auxin**	**Salicylic Acid**	**Cytokinin**
Fertilizer/Treatment	**----------------mg kg^{-1}----------------**			**-----------------kg ha^{-1}-----------------**		
Cyano-fertilizer	6.50E-05	5.92E-03	n/d	2.07E-04	0.02	n/d
Hydrolyzed fish fertilizer	3.97E-04	0.018	n/d	1.34E-06	2.59E-04	n/d
Non-hydrolyzed fish fertilizer	1.436	0.077	n/d	1.97E-03	2.47E-05	n/d
Seacom PGR Seaweed*	0.802	48.17	400**	9.62E-07	5.78E-05	4.80E-04
Neptune's Harvest Seaweed*	n/d	n/d	n/d	n/d	n/d	n/d

* seaweed N content = 0, applied at manufacturer's recommendation

** According to manufacturer. No cytokinin isomers were detected in phytohormone assay.

n/d – none detected

Table 3. Existing soil inorganic N conditions. The following inorganic N concentrations were present in the soil before planting carrots and cucumbers. Samples were taken to 58.8 cm. Results were based on a composite sample collected from plots in Fort Collins, CO. Samples were taken on April 10th, 2014 and May 9th 2015.

	Preseason inorganic N		
Year	NO_3^--N	NH_4^+-N	Total N
	-------------kg ha^{-1}-------------		
2014	19.6	30.8	50.4
2015	8.5	50.2	58.7

**1.2 kg m^{-3} is the assumed bulk density to convert from mg kg^{-1} to kg ha^{-1}*

Table 4. Nitrogen (N) fertilizer applications to carrots. Dates of applications, and individual and season fertilization totals by treatment type are shown. Applications were made to carrots in the 2014 and 2015 growing seasons in Fort Collins, CO.

	Soil N Fertilizer Applied		
	Cyano-Fertilizer	**Hydrolyzed Fish Fertilizer**	**Non-hydrolyzed Fish Fertilizer**
	---kg ha^{-1}---		
Application Dates		**2014**	
6/6/2014	4.3	4.0	4.5
7/11/2014	7.9	7.9	7.9
8/1/2014	9.9	9.9	9.9
8/11/2014	26.6	26.6	26.1
8/18/2014	19.7	19.7	19.3
Season Total	**68.4**	**68.1**	**67.7**
		2015	
6/26/2015	9.7	9.6	9.7
7/3/2015	3.0	3.0	3.0
7/17/2015	7.6	7.6	7.6
7/23/2015	8.1	8.1	8.1
7/30/2015	0.0	10.7	10.7
8/7/2015	0.0	10.7	10.7
8/21/2015	0.0	32.2	32.2
Season Total	**28.3**	**82.0**	**82.0**

Table 5. Nitrogen (N) fertilizer applications to cucumbers. Dates of nitrogen fertilizer applications and season fertilization totals by treatment type are shown. Applications were made to cucumbers in the 2014 and 2015 growing seasons in Fort Collins, CO.

Application Dates	Soil N Fertilizer Applied		
	Cyano-Fertilizer	Hydrolyzed Fish Fertilizer	Non-hydrolyzed Fish Fertilizer
	-----------------------------kg ha^{-1}-----------------------------		
		2014	
7/11/2014	7.9	7.9	7.9
8/1/2014	9.9	9.9	9.9
8/11/2014	26.6	26.6	26.1
8/18/2014	19.7	19.7	19.3
9/6/2014	12.6	12.6	12.6
Season Total	**76.7**	**76.7**	**75.8**
		2015	
6/26/2015	7.9	7.9	7.9
7/3/2015	2.4	2.4	2.4
7/17/2015	6.3	6.3	6.3
7/23/2015	6.6	6.6	6.6
7/30/2015	0.0	17.6	17.6
8/7/2015	0.0	17.7	17.7
Season Total	**23.2**	**58.5**	**58.5**

Table 6. Foliar seaweed applications to carrots. Season application dates and totals are shown. Applications were made to carrots in the 2014 and 2015 growing seasons in Fort Collins, CO. PGR seaweed is described as concentrated and had a greater dilution rate, thus the difference in product volumes. Both seaweed products were applied at the manufacturer’s recommended rates.

	Seaweed Type and Application Rates
	------------------------L ha^{-1}------------------------
Application Dates	**2014: Seacom PGR Seaweed**
6/3/2014	1.2
7/8/2014	1.2
7/29/2014	1.2
Season Total	**3.6**
	2015: Neptune's Harvest Seaweed
6/26/2015	31.7
7/3/2015	31.7
7/17/2015	47.5
8/7/2015	47.5
8/21/2015	63.4
Season Total	**221.9**

Table 7. 2014 ANOVA test results for carrots. N treatment and foliar seaweed effects on carrots grown in Fort Collins, CO in 2014 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments. * = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic							
Variable	Leaf Mg	Root Diameter	Root Length	Root Knobs	Undeveloped Roots	Post-Season Soil NO_3^--N	Stress	Yield
N treatment	3.85**	1.64	1.71*	2.96**	3.53**	3.78**	2.14	3.06**
Foliar seaweed	1.65	0.57	0.19	3.84*	1.42	0	3.99*	1.97
N treatment*Foliar seaweed interaction	0.84	2.56*	0.55	1.76	3.53**	0.39	5.40***	0.69

Table 8. 2015 ANOVA test results for carrots. N treatment and foliar seaweed effects on carrots grown in Fort Collins, CO in 2015 are shown. * = p<0.10, ** = p<0.05, *** = p<0.01. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments.

	F-test Statistic
Variable	Yield
N treatment	2.16*
Foliar seaweed	0
N treatment*Foliar seaweed interaction	0.14

Table 9. 2014 carrot length. Average length of 10 center carrots grown in Fort Collins, CO in 2014 is shown. Means that are statistically similar have a common letter, and means with differing letters are significantly different from each other ($p<0.05$).

N Fertilizer	Average Carrot Length
	--------------- cm ---------------
Control	21.74 B
Cyano-Fertilizer	23.43 A
Hydrolyzed Fish Fertilizer	22.78 AB
Non-hydrolyzed Fish Fertilizer	22.73 AB

Table 10. 2014 carrot stress. Average stress index of carrots grown in Fort Collins, CO in 2014. Stress was measured using an original scale from 0 (healthy plant) to 5 (dead plant). Means that are statistically similar have a common letter, and means with differing letters are significantly different from each other ($p<0.05$).

Treatment Combination	Stress Index
	---points---
Control	1.0 B
Control + S	0 B
Cyano-fertilizer	0.3 B
Cyano-fertilizer + S	0 B
Hydrolyzed Fish Fertilizer	0 B
Hydrolyzed Fish Fertilizer + S	2.5 A
Non-Hydrolyzed Fish Fertilizer	0.3 B
Non-Hydrolyzed Fish Fertilizer + S	1.75 A

Table 11. 2014 post-season soil NO_3^--N for carrots. Average soil NO_3^--N remaining in the soil after harvest in Fort Collins, CO in 2014. N was measured in mg kg^{-1} of NO_3^{--}-N to a depth of 58.8 cm. All of the N fertilizers (A) left behind more residual N than the control group (B). Soil NO_3^{--}-N was measured at the Colorado State University EcoCore after a 2M KCl extraction. Means that are statistically similar have a common letter, and means with differing letters are significantly different from each other ($p<0.05$).

N Fertilizer	**Post-harvest soil NO_3^--N**
	------mg kg^{-1}------
Control	6.6 B
Cyano-fertilizer	9.6 A
Hydrolyzed Fish Fertilizer	10.0 A
Non-hydrolyzed Fish Fertilizer	10.5 A

Table 12. 2014 ANOVA test results for cucumbers. N treatment and foliar seaweed effects on cucumbers grown in Fort Collins, CO in 2014 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments. * = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic		
Variable	Post-Season Soil NO_3^{-}N	Number of Cucumbers Row^{-1}	Yield
N treatment	4.18**	2.61*	2.64*
Foliar seaweed	0	0	0.15
N treatment*Foliar seaweed interaction	0.93	1.34	1.25

Table 13. 2015 ANOVA test results for cucumbers. N treatment and foliar seaweed effects on cucumbers grown in Fort Collins, CO in 2015 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments. * = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic			
Variable	Cucumber Diameter (Second Harvest)	Cucumber Length (Second Harvest)	Number of Cucumbers Row^{-1} (Second Harvest)	Yield (Second Harvest)
N treatment	0.88	0.21	3.00**	2.79*
Foliar seaweed	5.44**	3.60*	2.67	0.77
N treatment*Foliar seaweed interaction	1.09	0.95	1.44	1.31

Table 14. Cucumber harvest numbers and yield. Average number and yield of marketable cucumbers produced per treatment in Fort Collins, CO in 2014 and 2015 are shown. There were no significant differences in the first harvest of 2015. Means that are statistically similar have a common letter, means with differing letters are significantly different from each other. If all means did not significantly differ, no letter follows the mean ($p<0.05$).

	First Harvest		Second Harvest	
N Fertilizer	**Number of Cucumbers Row^{-1}**	**Yield**	**Number of Cucumbers Row^{-1}**	**Yield**
2014 Season		---kg ha^{-1}---		---kg ha^{-1}---
Control	10 AB	6,063 AB	--	--
Cyano-fertilizer	6 B	3,079 B	--	--
Hydrolyzed Fish Fertilizer	10 AB	6,366 A	--	--
Non-hydrolyzed Fish Fertilizer	12 A	7,114 A	--	--
2015 Season				
Control	4	3,630	10 A	18,205 A
Cyano-fertilizer	6	2,812	10 A	18,788 A
Hydrolyzed Fish Fertilizer	6	2,073	10 AB	12,395 B
Non-hydrolyzed Fish Fertilizer	5	1,726	9 B	11,866 B

Table 15. Soil media mix test results. Soil media contents (by volume), water holding capacity, and water loss after 3 days are shown. Soil mixtures were created and 500 mL of water were added in the spring of 2015 in Fort Collins, CO. The 60, 20, 20 mixture was selected because it held the second most water, and lost the second least moisture after 3 days.

Soil Components				
Sand	**Perlite**	**Vermiculite**	**Water Absorbed**	**Water Loss**
----------- % -----------			----- mL -----	----- % -----
50	37.5	12.5	132	1.62
50	25	25	119	1.68
40	30	30	114	1.98
60	**20**	**20**	**130**	**1.46**
60	30	10	127	1.96
33	33	33	122	1.01

Table 16. Nitrogen (N) fertilizer applications to peppers. Dates, N application rate, and total diluted volume of fertilizer are shown. Fertilizer was applied to potted bell peppers in Fort Collins, CO in 2015.

Date	**Soil N Fertilizer Applied**	**Volume of Fertilizer Mix**
	-------------- kg ha^{-1} --------------	------------ L pot^{-1}------------
2/28/2015	0.89	0.10
3/13/2015	1.69	0.10
3/23/2015	0.40	0.20
3/30/2015	3.71	0.20
4/8/2015	3.33	0.20
4/17/2015	4.02	0.30
4/28/2015	4.10	0.30
5/7/2015	1.23	0.30
5/14/2015	2.34	0.40
5/19/2015	1.61	0.40
5/26/2015	2.79	0.40
6/5/2015	1.60	0.40
6/12/2015	2.10	0.40
6/19/2015	2.12	0.15
Totals	**31.92**	**3.85**

Table 17. Foliar seaweed applications to peppers. Dates and total volume of PGR seaweed and Neptune's Harvest seaweed applied to bell peppers in Fort Collins, CO in 2015 are shown.

Date	**Volume of Seaweed Solution Applied**
	---------------------- mL ----------------------
3/3/2015	5
3/20/2015	10
4/16/2015	20
5/7/2015	20
5/28/2015	25
6/19/2015	25
Total	**105**

Table 18. 2015 ANOVA test results for peppers. F-test statistics for comparisons of means for peppers grown in Fort Collins, CO in 2015 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments.* = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic								
Variable	Abscisic Acid	Leaf Ca	Leaf Mg	Leaf Mn	Leaf Mo	Leaf N	Leaf S	Leaf Zn	Leaf OPDA
N treatment	2.26*	4.23**	2.51*	8.01***	9.08***	3.27**	2.80*	3.17**	1.52
Foliar seaweed	0.07	0.71	0.31	0.38	0.48	0.37	0.19	0.69	1.04
N treatment*Foliar seaweed interaction	0.67	0.24	0.16	0.19	0.49	2.17*	2.01	1.45	0.26

Table 18 Continued. 2015 ANOVA test results for peppers. F-test statistics for comparisons of means for peppers grown in Fort Collins, CO in 2015 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments.* = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic						
Variable	Branch to Height Ratio	Alive Flowers (Day 109)	Dead Flowers (Day 109)	Dead Flowers (Day 87)	Dead Flowers (Day 126)	Leaf Number (Day 67)	% Abscissions (Harvest)
N treatment	0.14	2.86*	2.40*	0.44	5.65***	3.08**	2.59*
Foliar seaweed	3.09*	1.72	1.31	3.93**	0.133	0.91	0.52
N treatment*Foliar seaweed interaction	3.94***	1.76	0.59	1	1.14	1.16	0.88

Table 18 Continued. 2015 ANOVA test results for peppers. F-test statistics for comparisons of means for peppers grown in Fort Collins, CO in 2015 are shown. Due to the large number of variables tested and the few significant differences, only significant results are displayed, variables not displayed were not significantly different among treatments.* = p<0.10, ** = p<0.05, *** = p<0.01.

	F-test Statistic						
Variable	Stress (Day 97)	Stress (Day 67)	Stress (Day 145)	Branch Number	Pepper Color	Pepper Shape	Yield
N treatment	3.24**	3.36**	4.36*	3.98**	0.45	0.05	2.36*
Foliar seaweed	0.12	0.23	0.47	4.00**	4.53**	2.67*	1.21
N treatment*Foliar seaweed interaction	1.04	1.97	0.56	2.04	1.88*	0.93	0.76

Table 19. Nutrient concentrations in pepper leaves. The nutrient concentrations found in dried pepper leaves is shown. Peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015. Means that are statistically similar have a common letter within rows, and means with differing letters within a row are significantly different from each other. If all means were statistically similar, no letter follows the mean. Sufficiency levels are shown for pepper leaf tissue analysis except for Mo, which is shown for mature vegetables (American Agricultural Lab, 2013) (Voss, 1998).

	N Fertilizer Treatment				
Nutrient	**Control**	**Cyano-fertilizer**	**Hydrolyzed Fish Fertilizer**	**Non-Hydrolyzed Fish Fertilizer**	**Sufficiency Levels**
N (%)	0.98 B	1.1 AB	1.1 AB	1.2 A	2.5-4.0
P (%)	0.42	0.40	0.40	0.44	0.20-0.40
K (%)	4.67	4.67	4.43	4.65	1.75-4.00
S (%)	0.51 B	0.59 A	0.51 B	0.55 AB	0.20-0.75
Ca (%)	2.6 A	2.3 AB	1.9 B	2.0 B	0.75-2.00
Mg (%)	1.0 A	0.98 AB	0.86 B	0.88 B	0.20-0.75
B (mg kg^{-1})	155 AB	162 A	129 B	152 AB	5-75
Cu (mg kg^{-1})	12	11	10	11	5-50
Fe (mg kg^{-1})	903 A	462 AB	464 AB	196 B	20-500
Mn (mg kg^{-1})	167 A	107 B	111 B	107 B	20-300
Mo (mg kg^{-1})	0.7 A	0.6 A	0.4 B	0.4 B	0.5-5
Zn (mg kg^{-1})	48 A	44 AB	37 B	38 B	5-75

Table 20. Phytohormone concentrations in pepper leaves. Phytohormones detected in fresh bell peppers leaves are shown. Plants were grown in a greenhouse experiment in Fort Collins, CO in 2015. Means that are statistically similar have a common letter within rows, and means with differing letters are significantly different from each other. If all means were statistically similar, no letter follows the mean.

	N Fertilizer			
Phytohormone	**Control**	**Cyano-fertilizer**	**Hydrolyzed Fish Fertilizer**	**Non-Hydrolyzed Fish Fertilizer**
abscisic acid (pg/mg)	21.48 B	41.04 AB	76.27 AB	131.8 A
jasmonic acid (pg/mg)	271.3	266.7	418.0	302.4
12-oxo-phytodienoic acid (relative abundance)	294.6 AB	189.2 B	299.9 AB	350.0 A

Table 21. Pepper plant flower count. The number of flowers alive or dead on a given measurement day are shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015 (see Figure 9). Means that are statistically similar have a common letter within columns, and means with differing letters are significantly different from each other. If all means were statistically similar, no letter follows the mean.

	Flowers Alive or Dead on Measurement Day				
	Day 87		**Day 109**		**Harvest (Day 135)**
Treatment	**Alive**	**Dead**	**Alive**	**Dead**	**% Abscissions**
Control	5.8	0.8	2.3 AB	3.0 B	65.4 A
Cyano-fertilizer	5.7	1.4	2.2 AB	4.4 A	54.8 AB
Hydrolyzed fish fertilizer	5.1	0.9	3.7 A	2.6 B	51.8 B
Non-hydrolyzed fish fertilizer	6.3	1.6	1.8 B	5.0 A	55.6 AB

Table 22. Pepper stress. Stress points counted on given measurement days for bell peppers grown in a greenhouse experiment in Fort Collins, CO in 2015 are shown. Means that are statistically similar have a common letter within columns, and means with differing letters are significantly different from each other. If all means were statistically similar, no letter follows the mean.

	Stress Points on Measurement Day		
Treatment	**67**	**97**	**145**
Control	0.3 B	0.3 B	3.9 A
Cyano-fertilizer	0.3 B	0.7 AB	3.3 AB
Hydrolyzed fish fertilizer	0.5 B	1 A	2.9 B
Non-hydrolyzed fish fertilizer	1.1 A	0.9 A	3 B

FIGURES

Figure 1. Stress metric example. Carrot plant with measureable stress is pictured. Stress was scored on a scale of 0-5 with 0 being a healthy plant and 5 being a dead plant. The carrot plant in this figure received a score of 2.

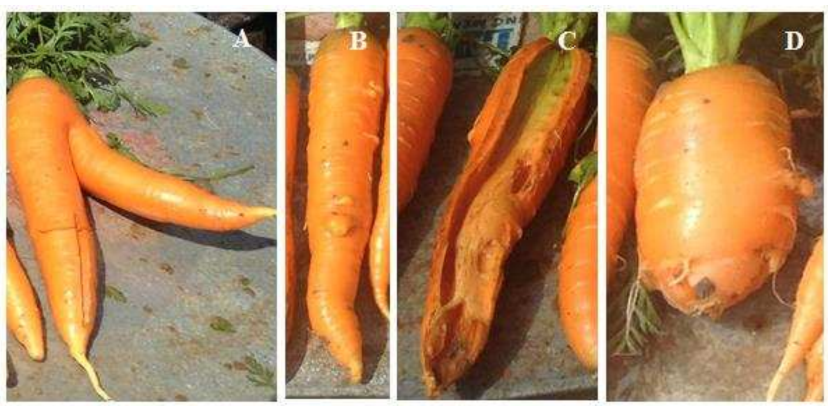

Figure 2. Carrot root deformities. Examples of marketability deformities that were quantified after carrot harvest in 2014 and 2015 in Fort Collins, CO are shown. The percentages of carrots displaying one or more deformities were compared among treatments. The deformities were described as branching (A), root knobs (B), cracks or splits (C), and underdeveloped root length (D).

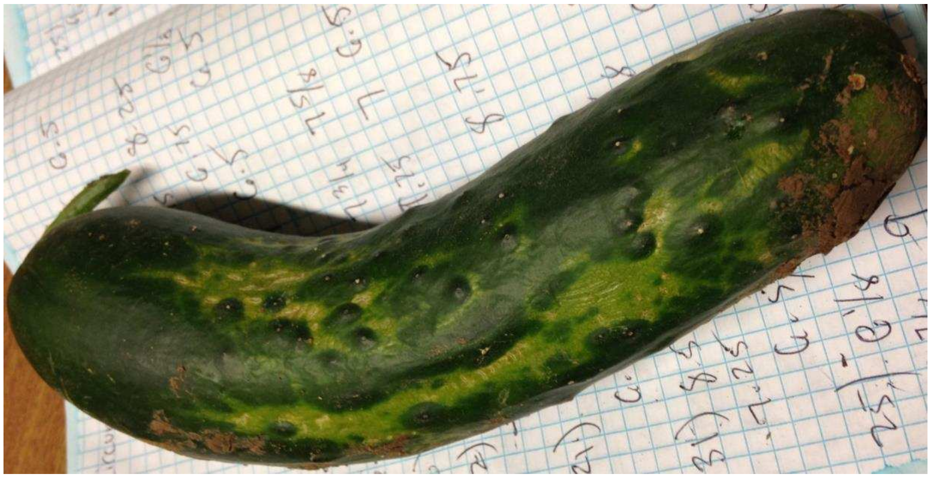

Figure 3. Example of an unmarketable cucumber. These cucumbers were discarded and were not counted toward row totals or total yield in field experiments in Fort Collins, CO in 2014 and 2015.

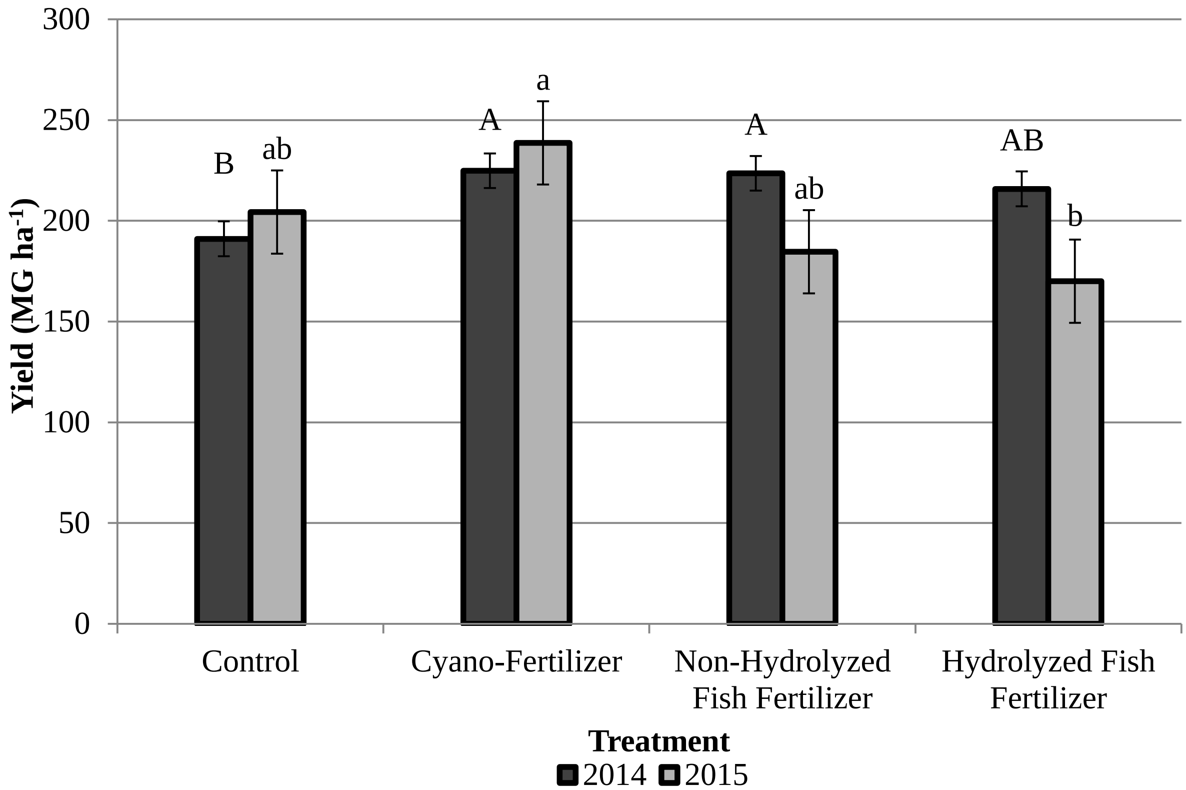

Figure 4. 2014 and 2015 carrot yield. Average yield of carrots in MT ha^{-1} was calculated by quantifying kg produced in row and between rows surrounding the center 10 carrots. Carrots were grown in a field experiment in Fort Collins in 2014 and 2015 is shown. Treatments that share a common capital letter are statistically similar. Treatments with different capital letters are statistically different from one another. Treatments that share a common lower case letter are statistically similar. Treatments with different lower case letters are statistically different from one another. The 2014 and 2015 seasons were not compared to each other ($p<0.05$).

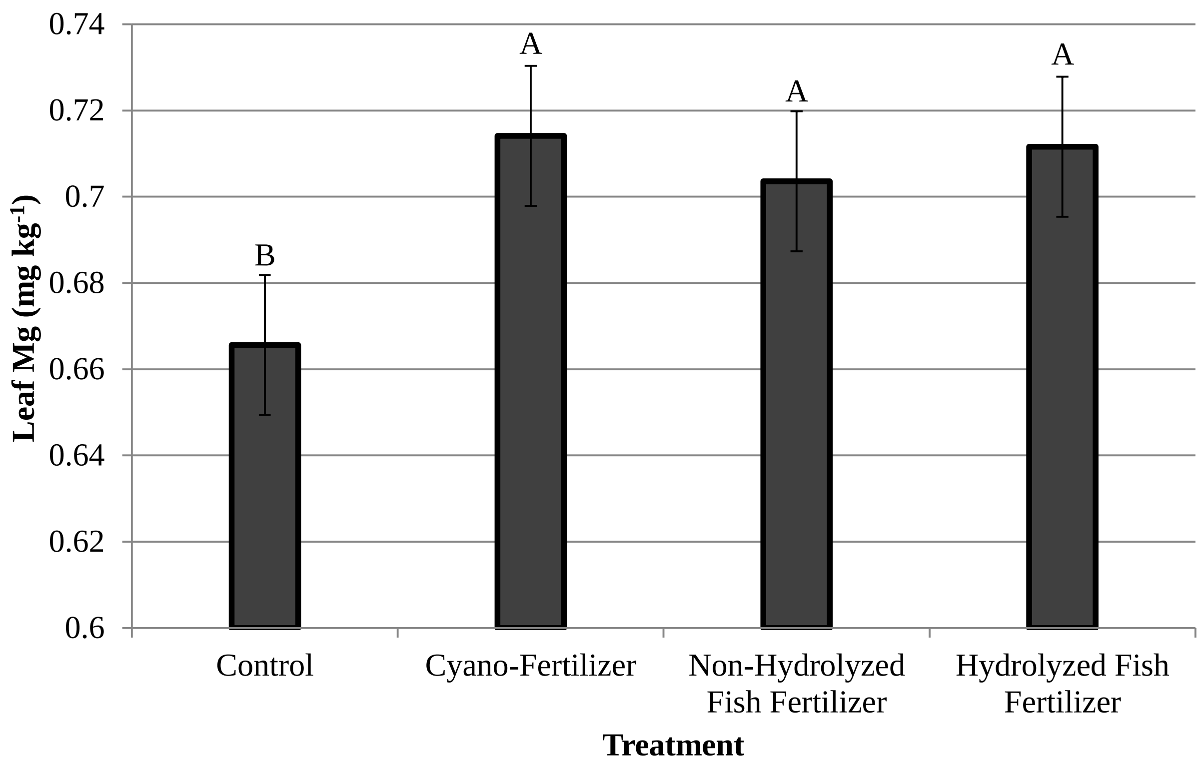

Figure 5. Carrot leaf Mg. Average leaf tissue Mg concentration (mg kg^{-1}) of carrot plants grown in 2014 in Fort Collins, CO is shown. Leaf tissue was analyzed by Ward Laboratories Inc. in Kearney, NE. Treatments that share a common letter are statistically similar. Treatments with different letters are statistically different from one another ($p<0.05$).

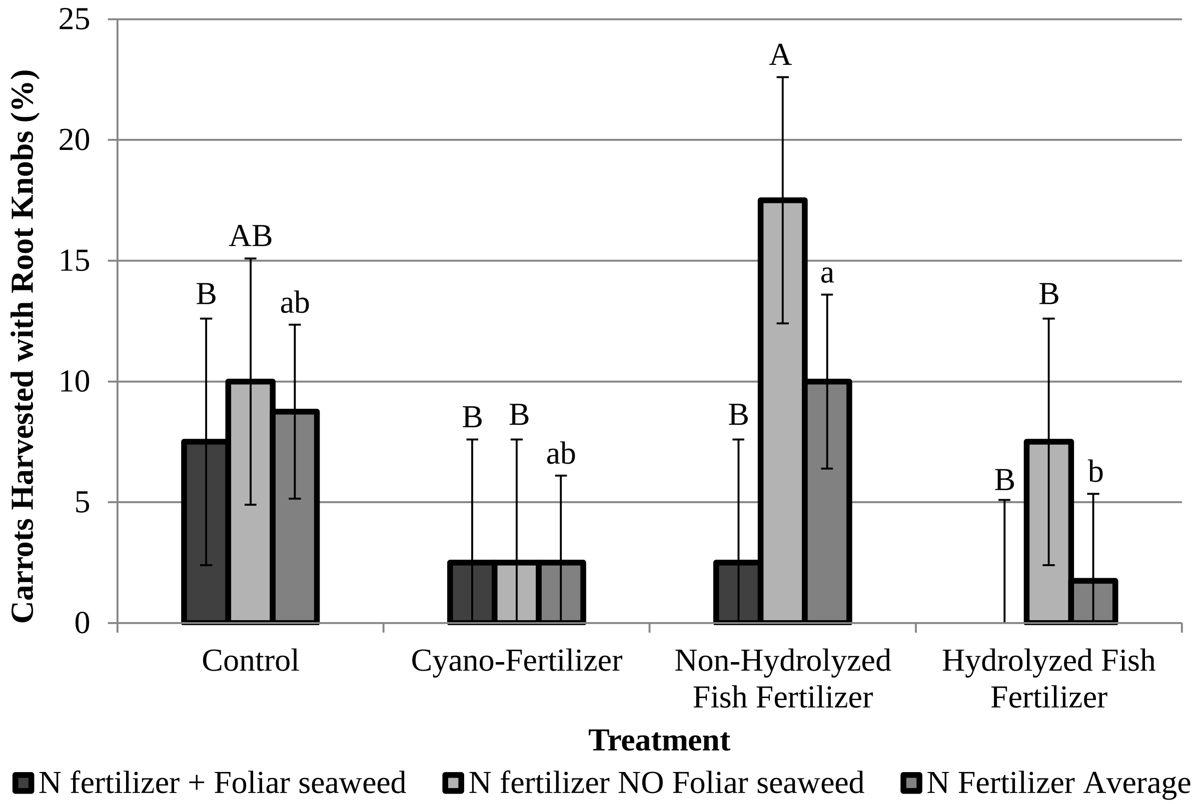

Figure 6. 2014 carrot root knobs. Average % of carrots grown in Fort Collins, CO in 2014 affected by root knobs that decrease marketability. Treatments that share a common letter are statistically similar. Treatments with different capital letters are statistically different from one another. Lower case letters are used for the N fertilizer averages because the average is not comparable to the N treatments with and without foliar seaweed ($p<0.05$).

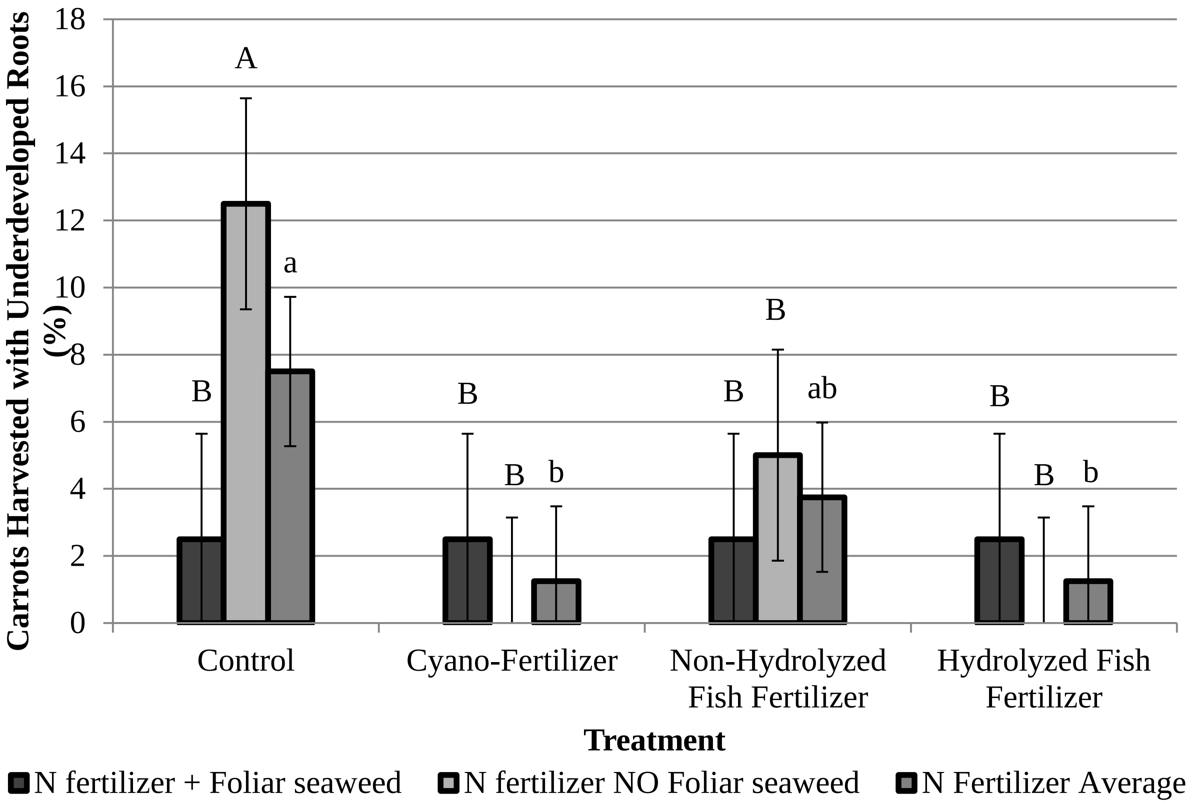

Figure 7. 2014 carrots with underdeveloped roots. Average % of carrots grown in Fort Collins, CO in 2014 affected by underdeveloped length that decrease marketability. Treatments that share a common capital letter are statistically similar. Treatments with different letters are statistically different from one another. Lower case letters are used for the N fertilizers averaged across seaweed treatments because the average is not comparable to the N treatments with and without foliar seaweed ($p<0.05$).

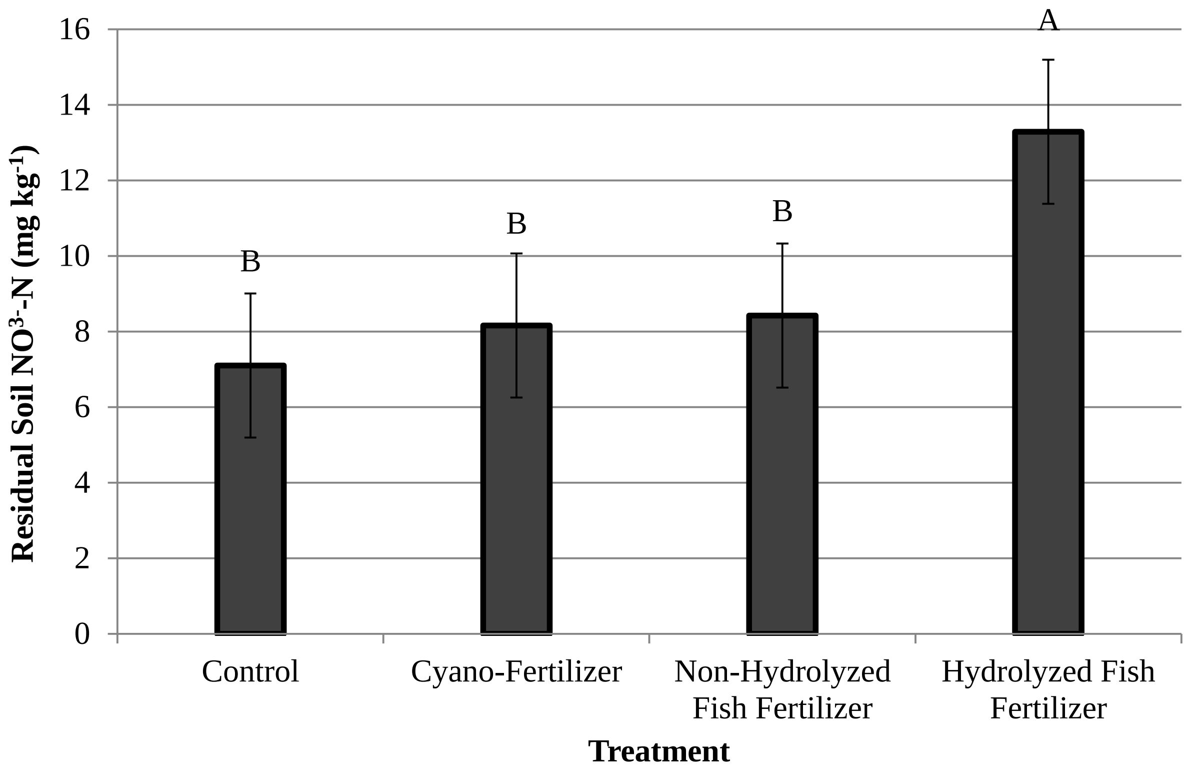

Figure 8. 2014 cucumber residual soil NO_3^--N. Remaining soil NO_3^--N (mg kg^{-1}) after a field experiment on cucumbers in Fort Collins, CO in 2014 is shown. Treatments that share a common letter are statistically similar. Treatments with different capital letters are statistically different from one another ($p<0.05$).

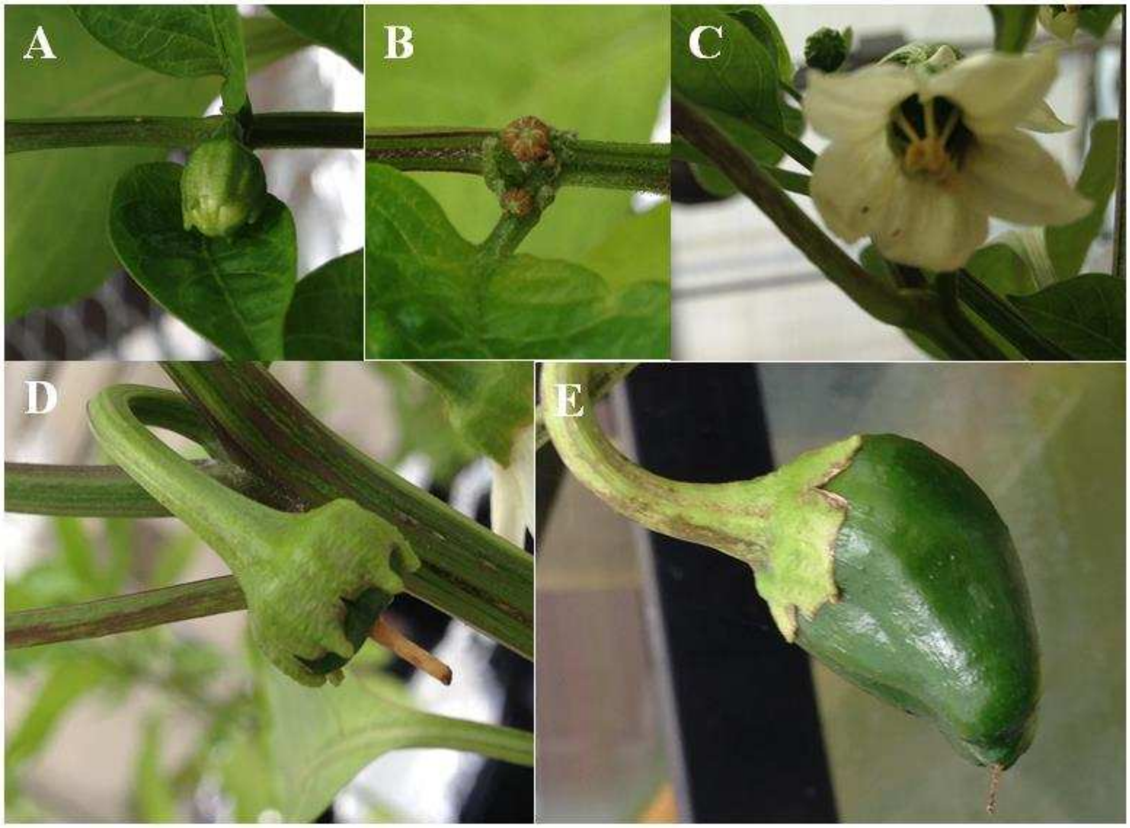

Figure 9. Examples of pepper flower classifications. The types of flowers present on bell pepper plants were counted each week and compared among treatments. The flower types fell into five categories: green flowers (A), dead flowers or abscissions (B), open flowers (C), finished flowers (D), and peppers (E). The photographs above were taken from the bell pepper plants grown in a 2015 greenhouse experiment in Fort Collins, CO.

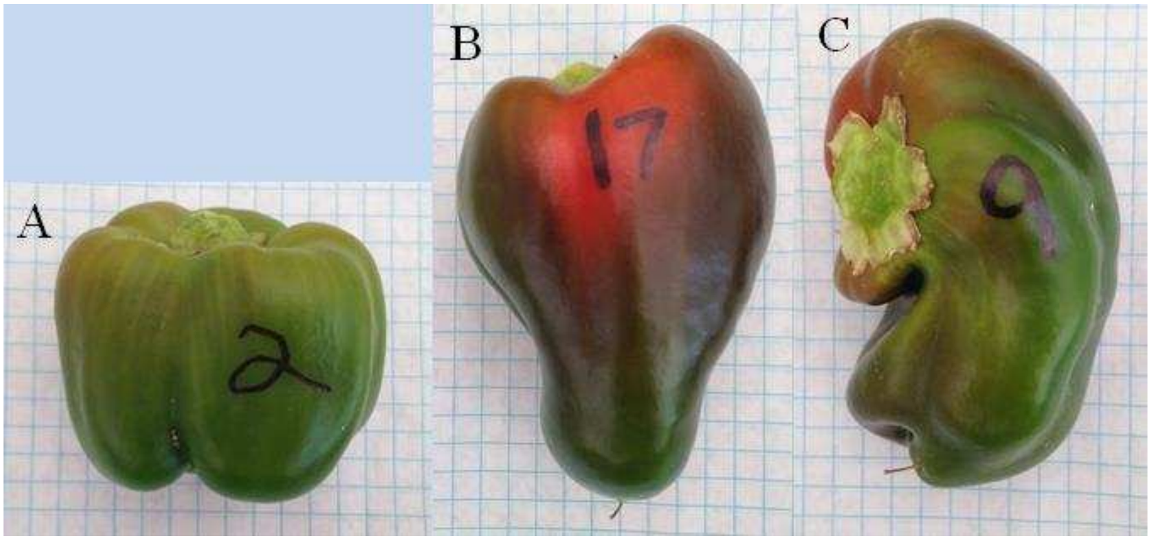

Figure 10. Examples of pepper fruit shape and colors. Three main shapes and two main colors of pepper fruit were observed in the greenhouse study. Incidence of these shape categories and colors were compared. The shape and color groups often overlapped. These peppers were classified as “green, bell shaped” (A), “red, long shaped” (B), and “green, curved” (C). Photographs above were taken from the bell peppers grown in a 2015 greenhouse experiment in Fort Collins, CO.

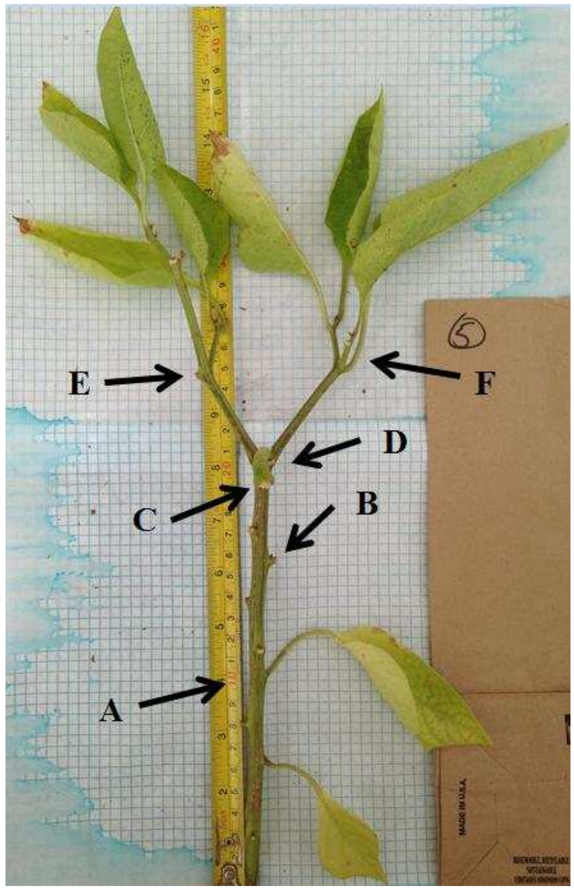

Figure 11. Example of pepper plant branch mapping. Measurements were performed on pepper plants upon harvest to investigate differences in branching characteristics. Events were measured in distance (cm) from the bottom of the stem. Events that were recorded were: locations of leaves (A), locations of abscissions (B), locations of fruits (C), distance to the first branch (D), and distance and numbers of secondary and tertiary branches (E and F).

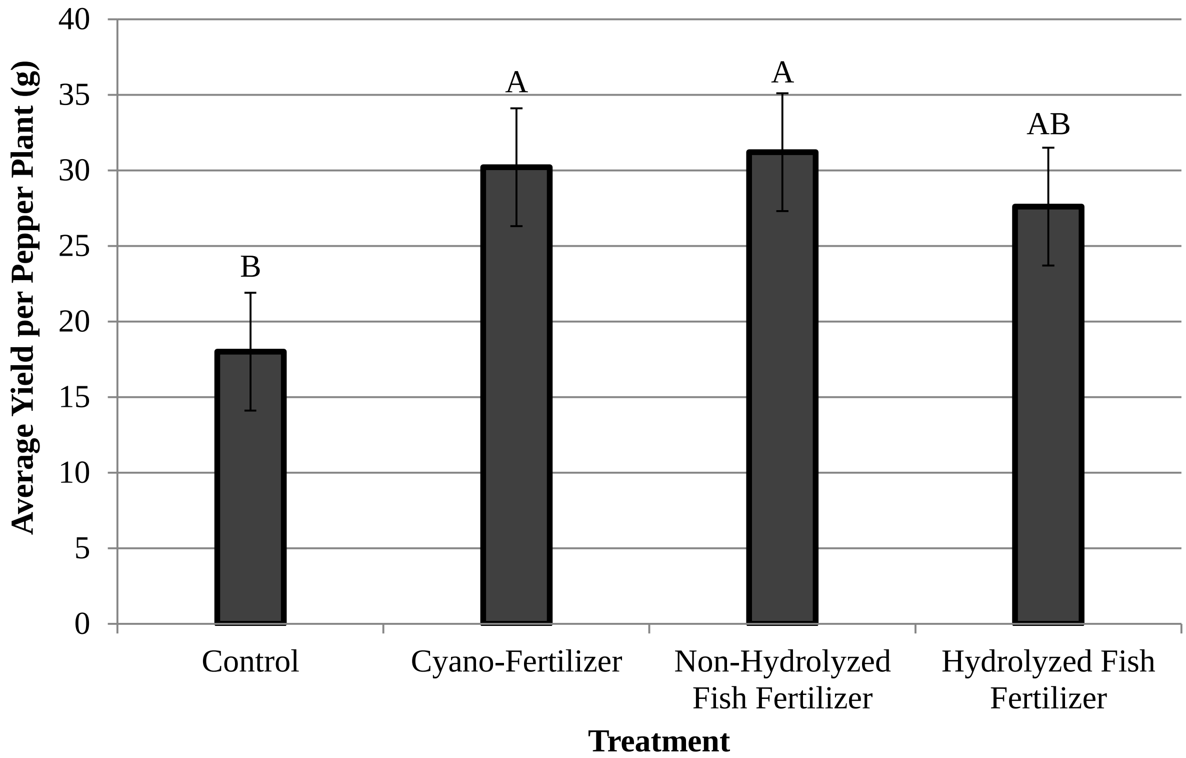

Figure 12. Pepper yield. The average fruit yield per plant (g) among N fertilizers treatments is shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015. Treatments that share a common capital letter are statistically similar. Treatments with different capital letters are statistically different from one another ($p<0.05$).

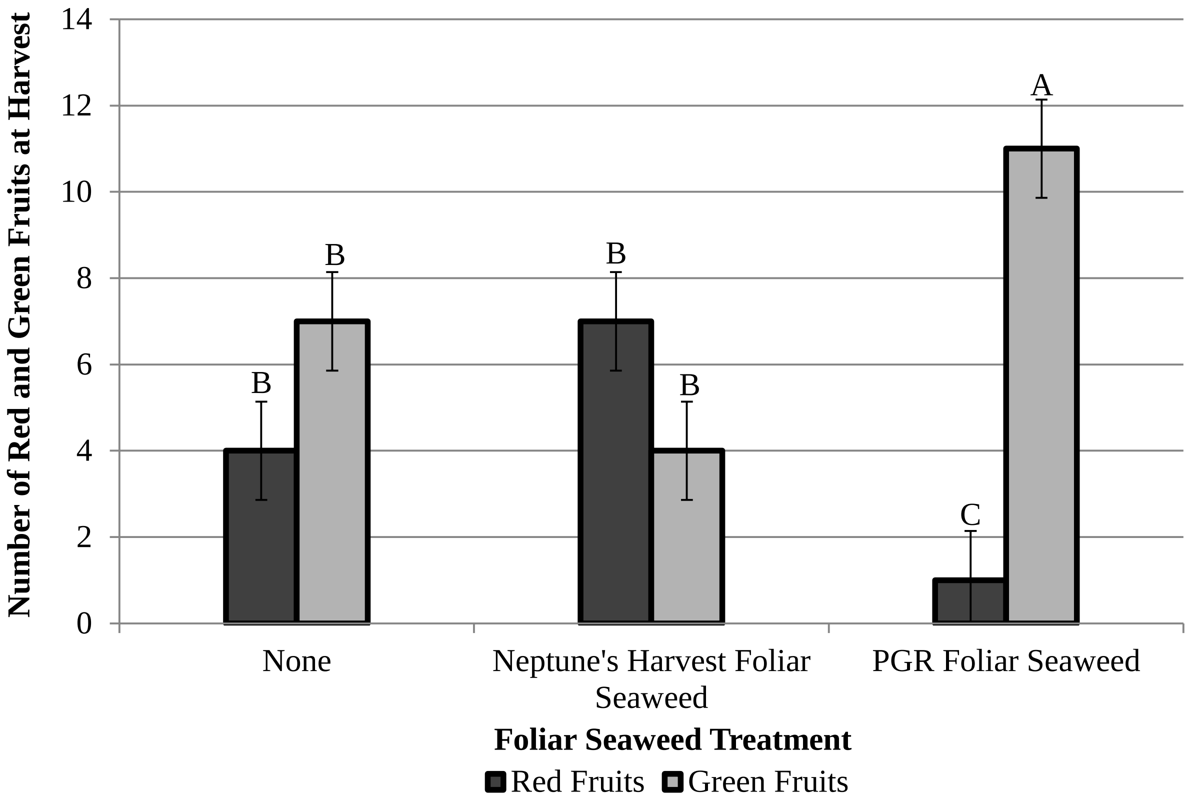

Figure 13. Pepper fruit colors. Differences in bell pepper color among foliar seaweed treatments are shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015. Treatments with different capital letters are statistically different from one another ($p<0.05$).

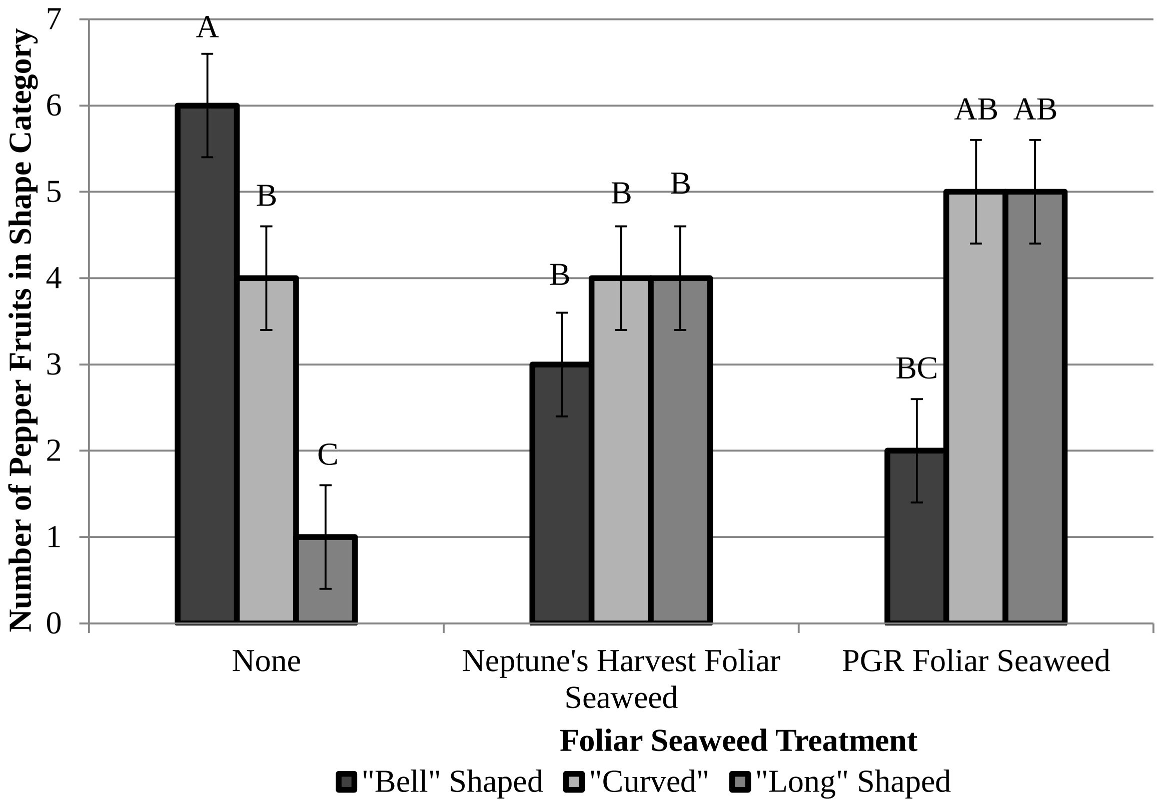

Figure 14. Pepper fruit shapes. Differences in bell pepper shapes among foliar seaweed treatments are shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015. The totals are given for number of "bell" shaped, "long" shaped, and "curved" shaped peppers produced (see Figure 10). Treatments that share a common letter are statistically similar. Treatments with different letters statistically different from one another ($p<0.05$).

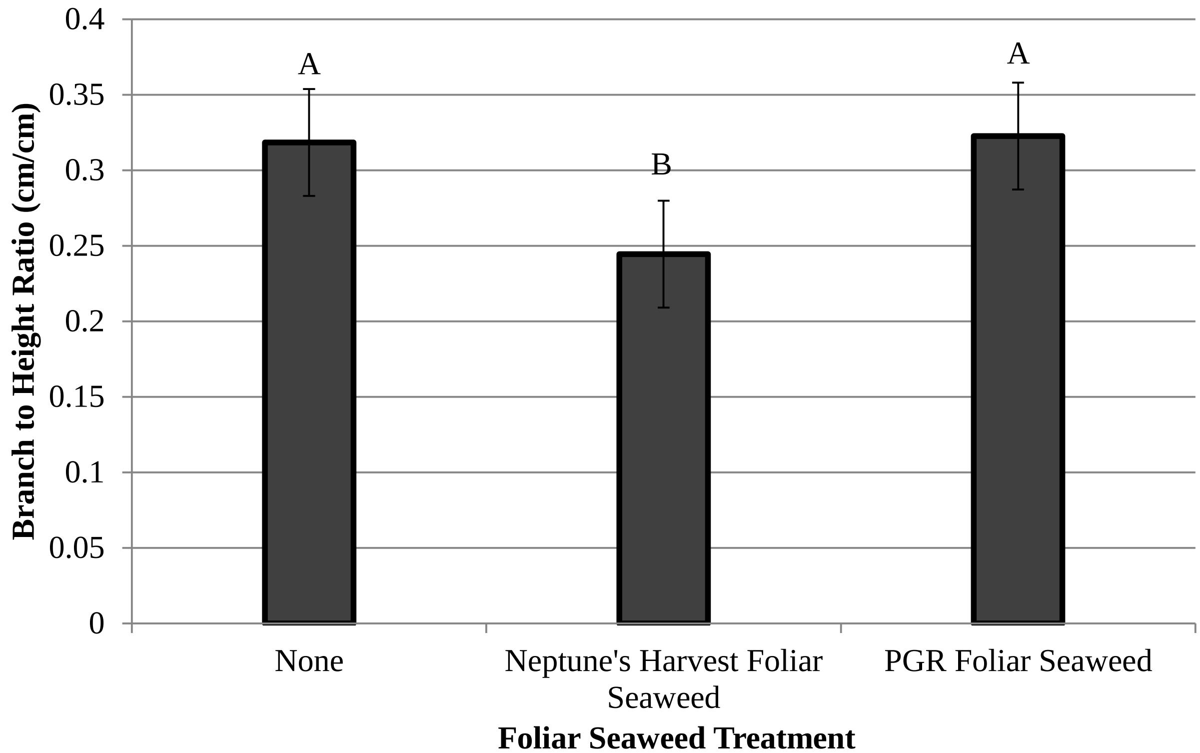

Figure 15. Pepper plant branch to total height ratio. The average branch height to total height ratio (cm) in bell pepper plants is shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 2015. Treatments that share a common letter are statistically similar. Treatments with different letters are statistically different from one another ($p<0.05$).

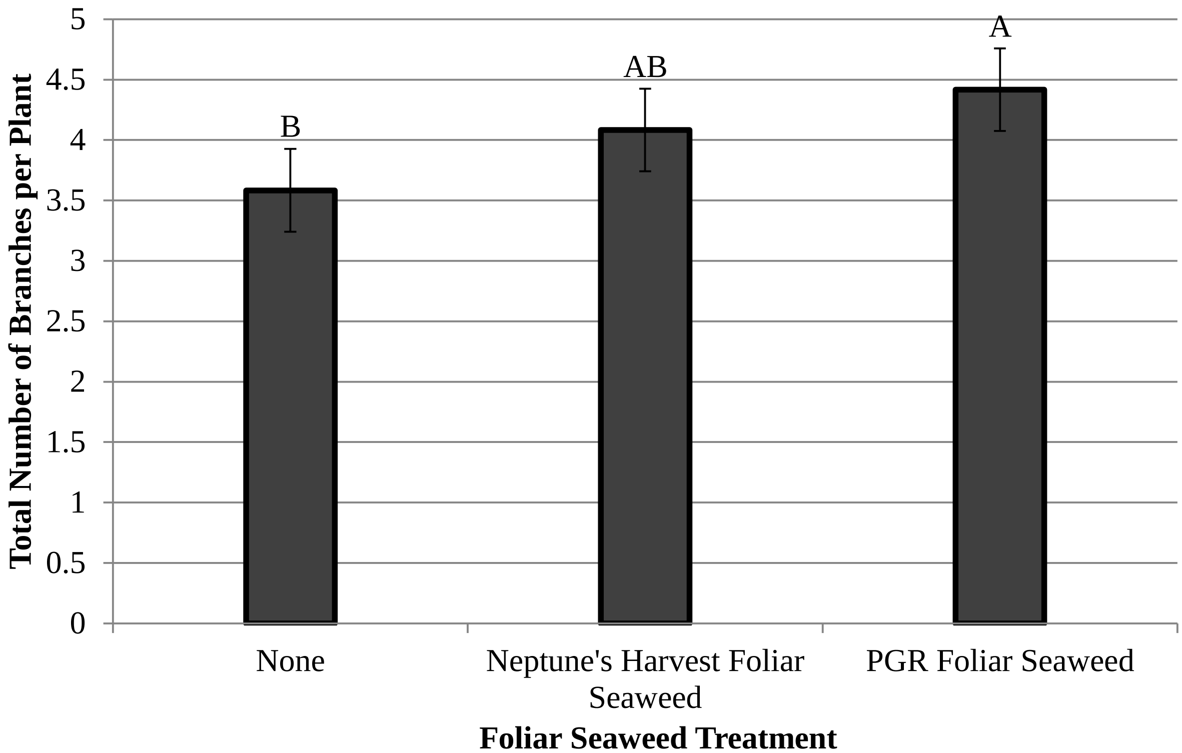

Figure 16. Total number of branches in pepper plants among foliar seaweed treatments. Average total number of branches in bell pepper plants is shown. Pepper plants were grown in a greenhouse experiment in Fort Collins, CO in 2015. Treatments that share a common letter are statistically similar. Treatments with different letters are statistically different from one another ($p<0.05$).

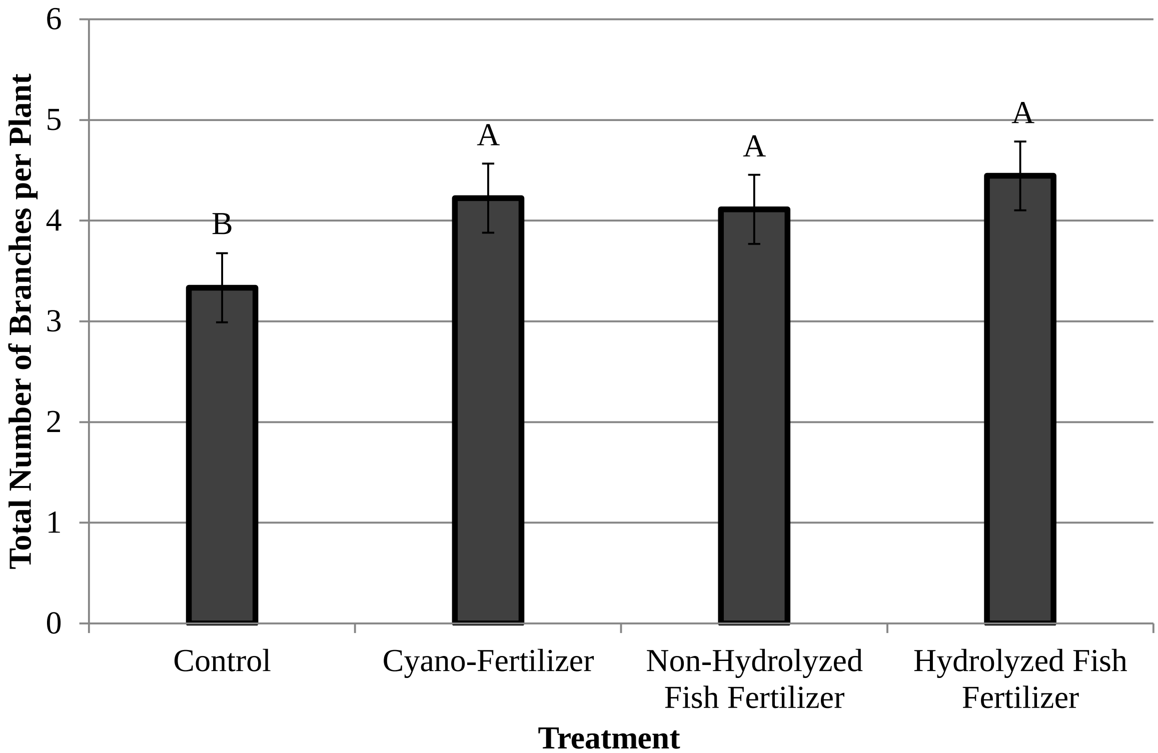

Figure 17. Total number of branches in pepper plants among N fertilizers. Average total number of branches in bell pepper plants is shown. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO in 215. Treatments that share a common letter are statistically similar. Treatments with different letters are statistically different from one another ($p<0.05$).

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