Exploration of Chufa (*Cyperus esculentus* L. var. *sativus* Boeck) as a novel specialty crop for the Northeastern United States

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ABSTRACT OF THE THESIS

Exploration of Chufa (*Cyperus esculentus* L. var. *sativus* Boeck) as a novel specialty crop for the Northeastern United States

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Thesis Director:

Dr. Mark G. Robson

Cyperus esculentus L. var*. sativus* (Boeck) is a globally-distributed sedge that produces edible tubers, commonly called nuts. The tubers are prized as a delicacy in parts of Nigeria and North Africa. This plant is known by many names, but is most commonly referred to as Chufa or Tiger Nuts. Chufa has been foraged as a part of the human diet during the Paleolithic Era, and has been cultivated in Egypt and North Africa for over 6000 years. We wanted to explore the potential of this specialty crop that is high in all 20 amino acids, in vitamins C and E, and in minerals, to evaluate the potential for growing it in the Northeastern United States. We also wanted to explore the optimal conditions for production for anyone who wishes to utilize this crop. We have evaluated the effects of potassium, iron, water, and growth media on the productivity of Chufa. We have chosen potassium to increase tuber size, iron for overall plant productivity, water for drought tolerance, and growth media for overall plant productivity. We have found that increasing potassium with nitrogen and phosphorus increases overall plant productivity, but increased potassium alone does not significantly increase tuber size. We have found that iron has no effect on plant productivity. We have found that the productivity of Chufa increases significantly with increasing water. We have found that professional growing medium is best for overall plant production, followed by organically fertilized organic soil. We hope that our findings help to make Chufa more accessible and enticing to the Western World as a health food crop.

Acknowledgement and Dedication

To everyone who believes in me.

I would also like to thank the guidance of my committee, Dr. Lena Struwe, Dr. Mark Robson, Dr. Albert Ayeni, and the support of the Department of Plant Biology and Pathology. With their patience, virtue, and leadership, this thesis is possible.

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Chapter 1 - Chufa, the plant, properties, and history

Cyperus esculentus var. sativus (Boeck) is a globally-distributed sedge that produces edible tubers, commonly called "nuts" (see Figure 1). The tubers have a nutty flavor, and both the plant and the tubers have been called by a wide variety of names including: Tiger Nuts, Tiger Nutsedge, Yellow Nutsedge, Chufa, Earth Almond, Earth Nut, and so on (Sánchez-Zapata, Fernández-López, & Pérez-Alvarez, 2012). It is a perennial that reproduces mainly asexually through tuberous growth. It rarely flowers, and when it does, most of the flowers are sterile (Sánchez-Zapata, Fernández-López, & Pérez-Alvarez, 2012). It grows up to about 0.3-0.8m in height, and prefers moist, loamy soil. The tubers taste similar to almonds and coconuts, and are prized as a delicacy in parts of Nigeria and North Africa (Bamishaiye, 2011).

Chufa has been cultivated for thousands of years, and since ancient times, has been grown in North Africa, especially Egypt (Negbi, 1992) and the surrounding area. The tubers have been found in the tombs of pharaohs of pre-dynastic times, about 6,000 years ago (Zohary, 1986), and are even thought to be a part of the diet of our Paleo-ancestors (Peters & Vogel, 2005). In fact, a recent Oxford University publication finds that indeed Chufa/Tiger Nuts were indeed a part of the diet of early man around the Pleistocene Epoch, and consisted of a large part of the diet (Macho, 2014).

The tubers are typically cultivated in soils rich in organic matter, and are light, like in the Nile Delta, or in the Valencia region in Spain. They are planted in raised beds, flooded for irrigation, and then, the aboveground shoots are burned when it's time to harvest.

The tubers do not get burnt because they are still underground. Tubers are harvested by either hand or a machine similar to a peanut harvester (Sánchez-Zapata, Fernández-López, & Pérez-Alvarez, 2012).

The tubers have an excellent dry weight nutritional profile, being high in protein (7% - 8.5%) (Codina-Torrella, Guamis, & Trujillo, 2014; Adejuyitan, 2011), high in lipids (28% - 35%) (Codina-Torrella, Guamis, & Trujillo, 2014; Adejuyitan, 2011), and high in energy (400 kcal/100g) (Codina-Torrella, Guamis, & Trujillo, 2014) compared to sweet potatoes, (100kcal/100g) another tuber. The tubers can be ground into flour to be used in baking products, and make an excellent wheat flour substitute for those with celiac disease, or looking for a gluten-free diet (Aguilar, Albanell, Miñarro, & Capellas, 2015; Ahmed & Hussein, 2014). The chufa oil shares a similar fatty acid profile to olive oil with high monounsaturated fatty acids (Arafat, Gaafar, Basuny, & Nassef, 2009; Ezeh, Gordon, & Niranjan, 2014). Oleic acid is the highest abundant fatty acid, and has been implicated in the reduction of heart disease, diabetes, and cancer (Amine, et al., 2002; Lunn & Theobald, 2006; Jones, et al., 2014). Vanillin has been found in roasted chufa oil, and is a favorable and marketable quality for the chufa oil (Ezeh, Gordon, & Niranjan, 2014). Chufa oil could serve as a natural alternate source of vanillin or aromatic food flavoring (Lasekan, 2013). Chufa tubers are also high in vitamins C and E (Belewu & Belewu, 2007). In addition to all these benefits, Chufa tubers also contain more than the adult FAO/WHO requirements for daily protein intake (Bosch, Alegria, & Farre, 2005), and more than the requirements for 17 out of 20 amino acids. Chufa tubers have low or zero amounts of asparagine, glutamine, and tryptophan (Bosch, Alegria, & Farre, 2005).

In North Africa, and in Spain, the tubers are also fermented and squeezed to produce *horchata de leche*, a popular beverage, which may be alcoholic or not. In other parts of the world, it is considered to be a persistent weed; although those weedy characteristics make it productive, and desirable to be used as a biofuel and other applications. Chufa has few pests and disease problems, making it hardy and desirable to be grown.

Iron Fortification

Iron is an essential nutrient in the human diet, and has many uses in the body. Iron is used as the center of the hemoglobin molecule, which is vital for oxygen transport from the lungs throughout the body. Iron is a component in myoglobin in the muscles, and is essential in enzymatic reactions and mitochondrial electron transport. Being so vital, deficiencies of iron are devastating, and if left untreated, will lead to gross malfunctions within the body, or death. According to the NIH, the Recommended Dietary Allowance (RDA) for Iron is 8-11mg (NIH, 2015). However, there are many areas in the world, including the United States, where it is hard to acquire the recommended amount through diet alone. According to the CDC, since the 1970's, iron deficiency as anemia has declined among children due to increased intake as infants, but anemic deficiency has still remained high for low-income women (MMWR, 1998).

Regulation of iron balance occurs in the gastrointestinal tract via absorption. Although the body generally holds onto the roughly 3g of iron, some does leave the body and must be replaced. This replacement is limited by the iron bioavailability and absorption in the gastrointestinal (GI) tract (MMWR, 1998). Iron uptake is influenced by many factors, such as the rate of red blood cell production, the rate of muscle development, respiration, the amount of iron stored in the body, the amount and type of iron in the diet, as well as inhibitors and enhancers in the GI tract and diet. The main controller of iron absorption is the GI tract, influenced by the total amount of iron in the body. If total iron is low, then the GI tract will increase absorption. Iron bioavailability can range as low as 1% when the body has enough, to 50% (Hallberg, 1981) when the body needs more. However, these percentages fluctuate depending on the aforementioned factors.

The bioavailability of iron is also greatly dependent on the food source. Plant-based iron, such as Chufa/Tiger nut iron, and iron-fortified foods, have iron available as non-heme iron. Animal-based (including meat, poultry, and fish) iron is available as heme iron, and is 2 to 3 times as readily absorbed as non-heme iron in the GI tract (Hallberg, 1981; Skikne, 1994). Enhancers of iron absorption include various acids, such as citric acid and ascorbic acid (Vitamin C). Inhibitors of iron absorption include tannins (from tea), phytates (from bran), and calcium (from dairy) (Bothwell, Overview and mechanisms of iron regulation, 1995) (Siegenberg, Baynes, & Bothwell, 1994).

In adults, about 1mg of iron is lost daily through sloughed-off skin and feces (Green, Charlton, & Seftel, 1968). Women of childbearing years require 0.3-0.5mg extra per day to compensate for menstrual loss (Bothwell & Charlton, Iron deficiency in women, 1981), and an extra 3mg per day while pregnant, and post-partum until iron levels have normalized (Hallberg, Iron balance in pregnancy, 1988). Excess iron is stored as either the soluble protein complex ferritin, or the insoluble protein complex hemosiderin. In healthy persons, about 70-80% of excess iron is stored as ferritin (Bothwell, Overview and mechanisms of iron regulation, 1995) (Bothwell, Charlton, Cook, & Finch, 1979).

However, iron deficiency is one of the most common maladies worldwide, with an estimated 1.5-2 billion people, which is *roughly a third of the entire world's population* (Miller, 2013) (Lynch, 2011) (MMWR, 1998). In the developing world, laborers with anemia have impaired work capacity, which may be reversible with treatment (Li, et al., 1994) (Cook, Skikne, & Baynes, 1994). Impaired labor capacity has economic implications, and if anemia is reversible with treatment, then treatment with foods fortified in iron would increase work capacity (Detzel & Wieser, 2015), which moves the economy.

Chufa makes underground tubers, and like any plant with underground storage tissues, it has an affinity for acquiring metals (Farrag & Fawzy, 2012) (Yadav & Chandra, 2011). Iron has been found to be one of the metals in the tubers, and has been measured in raw Tiger Nuts, and Tiger Nut flour. However, different groups have come up with different values, ranging from .65mg/100g to 4.1mg/100g of Tiger Nut flour (Chinma, Abu, & Abubakar, 2010) (Temple, Ojobe, & Kapu, 1990) (Oladele & Aina, 2007). We have found that the iron content ranges between 2.5 and 4.5mg/100g in both field and greenhouse conditions. This is about 30-50% of the RDA of iron in a completely reasonable snack (raw or roasted tubers) or meal size (baked goods from Tiger Nut flour).

However, to treat anemia, the lost iron must be replaced. Therefore, it is necessary to increase iron intake. Iron supplements are expensive in the developing world, and are not as available to the poor as fortified foods are. However, foods with added iron run the risk of going rancid sooner, having a strange taste, and costing a bit more (Pasricha, Drakesmith, Black, Hipgrave, & Biggs, 2013). It is more applicable to naturally increase the inherent iron in foods such as Chufa/Tiger Nuts, so as to not disrupt the food quality. In Africa, China, and Southeast Asia, food fortification has reached the broadest amount of people, and has had the greatest benefit at the least cost (Detzel & Wieser, 2015). The CDC recommends treating persons with anemia with daily 60-120mg of iron supplements. The equivalent in Chufa tubers (at 3mg/100g) would be 2000g, which is 2Kg or 4.4pounds of tubers. This is indeed a lot to consume to forego the iron pill. People around the world like variety in what they eat, and no matter how tasty a food is, it is unlikely that they will eat bowls and bowls (or 4 pounds!) of that food every day for a long period of time. Persons with anemia will likely follow that pattern, and will not eat bowls and bowls of tubers for the extended amount of time that their bodies need to accumulate iron to normal levels. Therefore, it is necessary to increase the iron contained within the tuber, ideally in the ferritin form.

Accumulation within the plant

Chufa is a sedge, within Cyperaceae, and in the order Poales. Chufa and various grasses reproduce by rhizomes and have similar pathways for dealing with nutrients (Nozoye, et al., 2011). As one of the most widespread plants in the entire world, the members of Poales have adapted to many environments. As such, they have many mechanisms for dealing with different environments. Chufa has been used as a bioaccumulator of heavy metals including iron in bioremediation projects (Yadav & Chandra, 2011). Like most graminaceous plants (*sensu lato*), Chufa does not absorb iron with the help of acids. Rather, Chufa makes proteins like phytosiderophores and release them into the soil (Morrissey & Guerinot, 2009), which scavenge iron as ferric. Chufa then absorbs the chelated proteins, and then convert the iron into ferrous iron via iron chelate reductase. Both the graminaceous plants and other types of plants release acids to prevent the oxidation of ferrous iron, and just absorb ferrous iron directly (Morrissey & Guerinot, 2009).

Perhaps a transgenic approach to the increase of iron would be to up-regulate the genes involved in the formation of the phytosiderophores (PS). AtIRT1 is a divalent metal transporter in Arabidopsis that transports ferrous iron from root to shoot. Loss of function mutants have seen iron accumulation in the roots (Vert, et al., 2002) (Connolly, Fett, & Guerinot, 2002). Perhaps this could be another approach to increasing tuber iron. In non-graminaceous plants, phenolic compounds are secreted into the rhizosphere, and Fe is taken and pooled in the apoplast. The apoplast, acting as an Fe reservoir, stores about 75% of Fe in the roots, in plaques on the negatively-charged cell walls, which are cation sinks (Roschzttardtz, et al., 2013) (Morrissey & Guerinot, 2009). The mechanism for this is relatively unclear, however, the Fe-nicotianamine (NA) transporter AtYSL3 would be a good candidate (Morrissey & Guerinot, 2009).

In graminaceous plants, iron take-up is dependent on siderophores with an affinity for ferric iron. Mugineic acid (MA) family PSs are synthesized from L-methionine and released from the root epidermis. In barley, the genes for sulfur uptake, methionine synthesis, and PS synthesis are dramatically up-regulated in the first 24h of iron deficiency (Roschzttardtz, et al., 2013) (Morrissey & Guerinot, 2009) (Nozoye, et al., 2011). In rice, TOM1, the efflux transporter of deoxymugineic acid (the primary phytosiderophore from rice and barley) is overexpressed upon iron deficiency. Coupled with the expression of the nicotianamine transporters, ENA1 and ENA2, this explains the main molecular mechanism in rice for iron acquisition from the soil (Nozoye, et al., 2011). Also, in rice, the expression of the transcription factor, OsIRO2 is upregulated, and is implicated in the up-regulation of the sulfur pathway and PSs (Itai, 2013). The FeIII-PS chelate complexes are taken up by the plant via a high-affinity uptake system, are not as influenced by pH than the reduction strategy, except that their secretion is positively linearly correlated with increasing pH.

Once inside the epidermis of the root, the Fe-PS complexes are reduced so that they release ferrous iron, which is taken up by unknown chaperones and proteins. The iron is then transported symplastically to the pericycle, where it enters the xylem and transported up to the leaves.

In the xylem, Fe is bound to citrate at lower pHs (~5.5), and to NA at higher pHs (~7.5) (Rellán-Álvarez, 2008). Fe complexes are important in the leaves for electron transport, enzyme cofactors, and photosynthesis. Citrate is important in the xylem, and another group (Guo et al., 2014) found that in another graminaceous plant, *Phragmites spp.*, exogenously applied citric acid has been found to significantly increase Fe concentration in the roots and rhizomes (Guo & Cutright, 2014). They have also found that the rhizosphere has little effect on Fe absorption. However, it could be surmised that bacteria which produce citric acid living in the soil could potentially increase iron in graminaceous plants, including Cyperaceae. Although not many bacteria and fungi in the soil may not interact directly with *Cyperus*, their role in “soil preparation” is relatively unknown. Bacteria and fungi may change soil conditions to be conducive to the absorption of Fe, or they may change soil conditions to be non-conducive to Fe. In another study, bacteria (*Bacillus* spp., *Streptomyces luteogriseus*, and *Pseudomonas fluorescens*), not fungi, were responsible for Fe and P accumulation within *Carex kobomugi*, another sedge by increasing availability in the soil (Matsuoka, Akiyama, Kobayashi, & Yamaji, 2013). Increasing beneficial bacteria in the soil related to iron-uptake may increase iron, and will appeal to the organic farming community.

Since Chufa number of shoots correlate positively with number of tubers (Figs 1-3), one could argue that increasing the number of shoots also increases number of tubers. One could also argue that increasing photosynthetic efficiency would increase production of the plant, although our data does not support the latter. Perhaps it would be through N-fertilization that higher tuber production and size would be recorded. Another study has found that N with K in a certain ratio increases tuber yield (Pascual, Maroto, LóPez-Galarza, Sanbautista, & Alagarda, 2000). This might be another way to increase biomass and yield.

Oil Applications

As our finite resource of crude oil runs lower and lower, the need for renewable sources of fuel increases. One of the more promising ways towards creating a sustainable renewable energy economy is through biofuels. Biofuels

Chufa has been little-explored for use in biofuels. The leafy aboveground structure grows quickly, is easily harvestable, and is hardy. When we did an analysis on the ash content of the leaves, we have found that the ash content is around 7%, which is too high for the recommended 3% or less to be suitable for biofuels. However, this was coming from fully-fertilized plants, and perhaps reduction of soil fertility might decrease the ash content in the leaves, but this has not been explored.

Decrease in fertility too much hurts the plant, so a golden-medium would have to be found. If so, then perhaps Chufa will be able to compete with other biofuels. Chufa has the advantage of not being picky about the ground in which it grows in. Most biofuel crops compete with actual crops for farmland space. It can be planted on hilly, fallow land that is unsuitable for conventional crops, where it would grow as wild anyway. This gives it a key advantage over other biofuel crops such as corn, which have specific field needs.

The tubers can be pressed for oil, and the oil is not only applicable to food, but also to use towards the production of biodiesel. In one study (Ofoefule, Ibeto, Okoro, & Onukwuli, 2013), the high and moderate flash points of the Chufa biodiesel and blends ranged between 90-178^{o}C, their cloud points ranged between 6.5-13^{o}C while their pour points ranged between -3-(-10) °C. General results of the blends have performance results closer to petro-diesel and ASTM standards. Thus, blends of Chufa-derived oil have been shown to be good for both biodiesel and engines and non-biodiesel engines.

In another study (Wang, Zhou, Liu, Li, & Zhang, 2013), to reduce the cost of algal-based biodiesel, *Cyperus esculentus* waste was used as the carbon source of the oleaginous microalgae *Chlorella vulgaris*. It demonstrated that *C. vulgaris* grew better in Chufa waste hydrolysate than in glucose medium under the same reducing sugar concentration. Chufa waste hydrolysate concentration influenced the cell growth and lipid production significantly. The produced biodiesel was analyzed by GC–MS and those results suggested that lipids produced from the Chufa waste hydrolysate has decent potential to be feedstock for biodiesel production.

Still, even if the oil were used for food alone, it has similar properties to olive oil- in that it is high in monounsaturated fatty acids, oleic acid, and other key components of olive oil (Ezeh, Gordon, & Niranjan, 2014) (Lasekan, 2013) (Sánchez-Zapata, Fernández-López, & Pérez-Alvarez, 2012).

Cultivars

Cultivars of Chufa have not been formally named or identified, most likely because Chufa is almost an exclusively asexually-reproducing plant. Dr. Albert Ayeni has acquired several lines of Chufa from markets on Ghana and Nigeria, as well as from Organic Gemini LLC., in Brooklyn, NYC. Perhaps from genetic mutation accumulation, or perhaps from epigenetic changes, we have identified several selections with discernable traits. GH, from Ghana, yields larger tubers that are scattered through the soil. MV, from Nigeria, yields oblong, sometimes branching tubers. SK, from Nigeria, grows in dense clusters, and yields smaller tubers lined along the surface. OG from Brooklyn behaves like GH, but was originally acquired from Spain. Genetic tests will need to be done to parse out cultivars.

Discussion

Overall, Chufa is a hardy plant with many applications. It has few pests, which make it desirable to grow, especially under organic conditions. Its tubers can be eaten raw, roasted, crushed into flour to make gluten-free products, pressed for oil similar to olive oil, and fermented into horchata beverages. However, as a root crop, the tubers ought to have a high affinity for minerals, which they do. But, to help make iron more available to poor communities, nutrition must be through diet, and that requires the bolstering of iron content within the tuber. This can be done by adjusting the microbes in the soil to facilitate iron acquisition, applying exogenous chelates in order to facilitate iron acquisition, or possibly genetically engineer the Tiger Nutsedge to increase acquisition of iron.

Another application would be to encourage community gardeners to plant this plant for its health benefits, as well as increase market awareness of Chufa/Tiger Nuts as a health food. Community gardens often donate their produce to food shelters, and Chufa tubers are a highly nutritious food that will benefit those at food shelters. Its impact on urban communities and urban gardening has not been studied. In fact, with little space requirements, it would be an ideal crop to grow in the cities, on the ground, in planters and balconies, and in windowsills. Its contributions to city greenspace would be noticed, as it is a hardy plant tolerant of pollution, and, like any plant, reduces CO_2 by providing a CO_2 sink. It can even be used to prevent erosion, as the thick mat of root fibers creates a net that traps soil and holds it in place. Chufa has a lot that it can do for cities such as NYC, and the surrounding areas.

Biofuels are a hot issue, and Chufa is a contender in the biofuels arena. However, work will need to be done to reduce the ash content of the leaves, though this is not impossible. Chufa seems to be most effective at providing a medium for biofuel-producing bacteria to use as a carbon source. Fermenting the leaves produces quality biofuel that can be used in many different types of engines. Coupled with the fact that this plant can produce quite a bit of biomass within a few months makes it competitive with other biofuel-producing plants.

Given all that this plant can do, we need to take advantage of this resource and use it. It is worthy of being studied and applied to solve our problems.

Figure 1 – Chufa and a view of the tubers beneath the soil- a photo from our study. Note the fibrous roots, as well as rhizomes that terminate in the edible tubers.

Chapter 2 – Characterization of Chufa: Potassium Enrichment of Soils

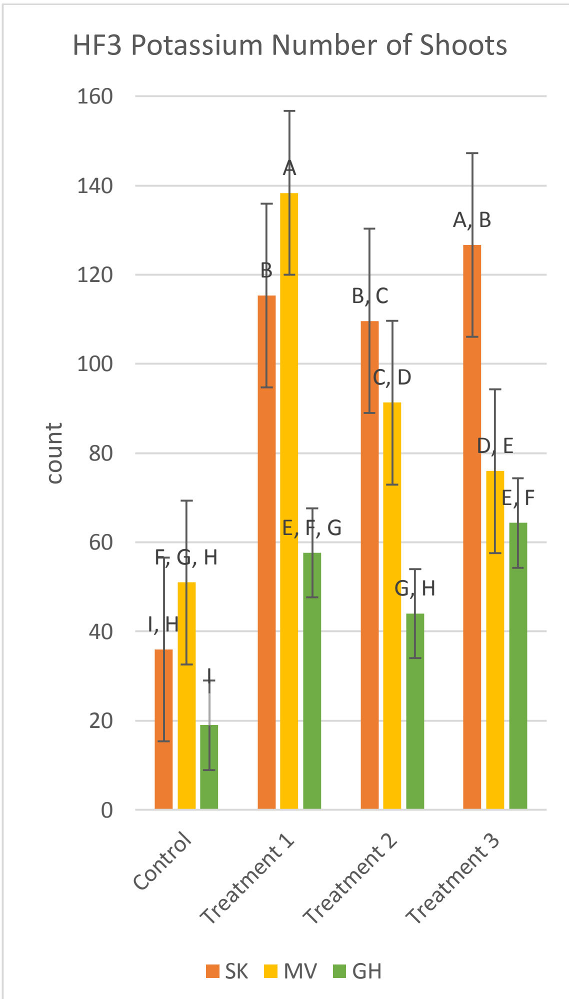
We had surmised that if potatoes responded to potassium application by making larger tubers (Chapman, Sparrow, Hardman, Wright, & Thorp, 1992) (Panique, et al., 1997), the Chufa ought to respond to excess potassium by making larger tubers as well. Thus, we designed an experiment to test if this is true. We wanted to determine the effects of potassium treatments on three selections of Chufa in two different environments- a climate-controlled greenhouse, and an open hoop-house.

Objective – To determine the effects of potassium applications on plant productivity in two different environments.

Methods –

Two experiments were planted during the week of April 27, 2014, and harvested 14 weeks later, during the week of July 21, 2014. One was planted in a hoop-house style greenhouse at 67 Ryders Lane, East Brunswick, NJ, known as Horticulture Farm 3 (HF3), the other was planted at 18 College Farm Road (CFR), New Brunswick, NJ in a permanent, climate-controlled greenhouse. Three different selections of Chufa were used- MV, SK, and GH. Selection germplasm was obtained from markets in Ghana and Nigeria. Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with Pro-Mix® brand BX general-purpose professional growing medium soil. We refer to it as GreenHouse Mixture (GHM) soil. Three plants were planted per flat, and each of the controls received only 10g of NPK fertilizer. Appended potassium fertilizer was added to experimentals in the form of muriate of potash (MOP). Trial 1 received 20g NPK/m^2. Trial 2 received 10g of NPK plus 10g MOP/m^2. Trial 3 received 10g of NPK plus 20g MOP/m^2. Trials were watered every day until soil was saturated. At CFR, temperature was kept constant at about 24-27°C. HF3 temperature remained the same as outdoor temperature. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber-formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed. Rooty fibers were detached and discarded. Tuber weight is the weight of all tubers produced in a flat (tuber yield weight). Results were statistically analyzed using the "proc glm" command in the SAS software suite. Fisher's Least Significant Difference (LSD) model was applied to the data ($p<0.05$), and significance groups were generated and applied to the results. The highest significance level was marked as "A", with lower significance levels marked as "B", "C", and so on.

Results –



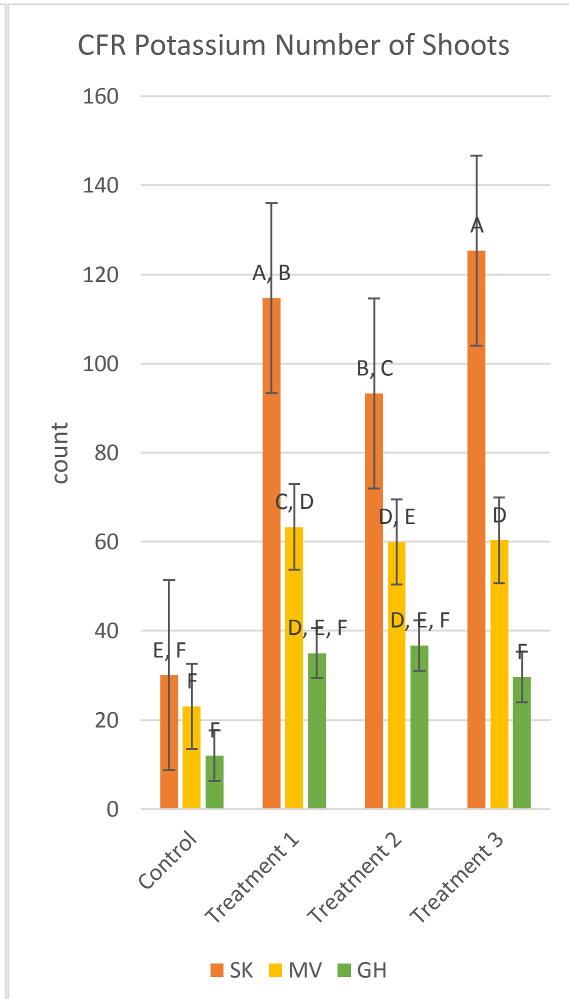
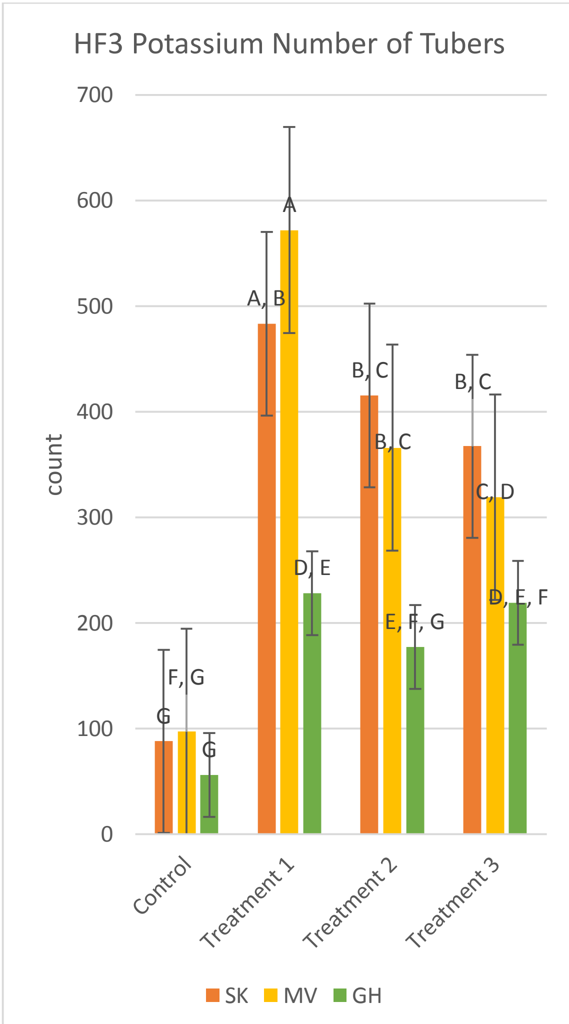


Figure 2 – The number of shoots produced by each flat at HF3. SK, MV, and GH are compared by treatments. All treatments yielded significantly more shoots than the control.

Figure 3 – The number of shoots produced by each flat at CFR. SK, MV, and GH are compared by treatments. All treatments yielded significantly more shoots than the control with the exception of GH.



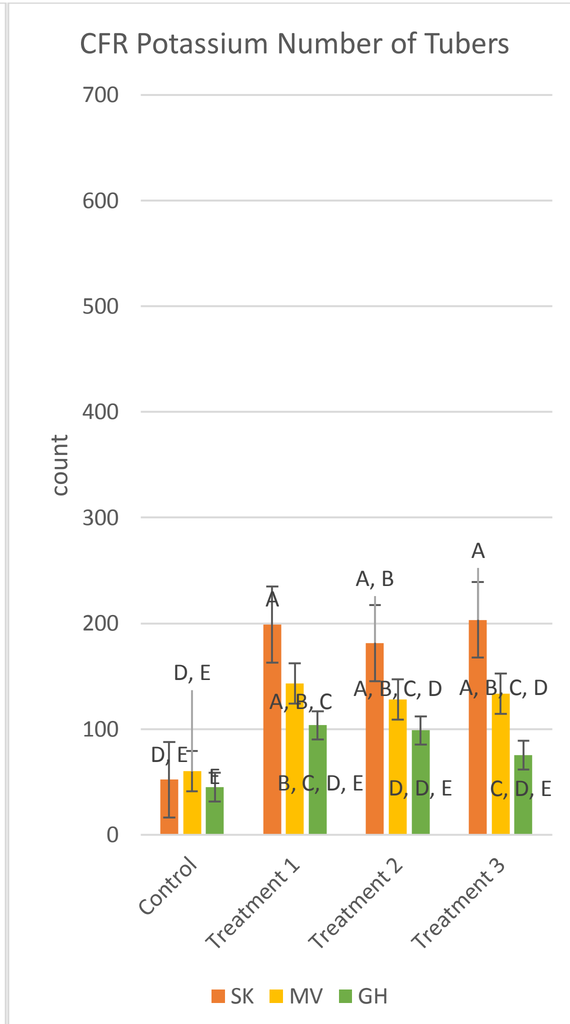
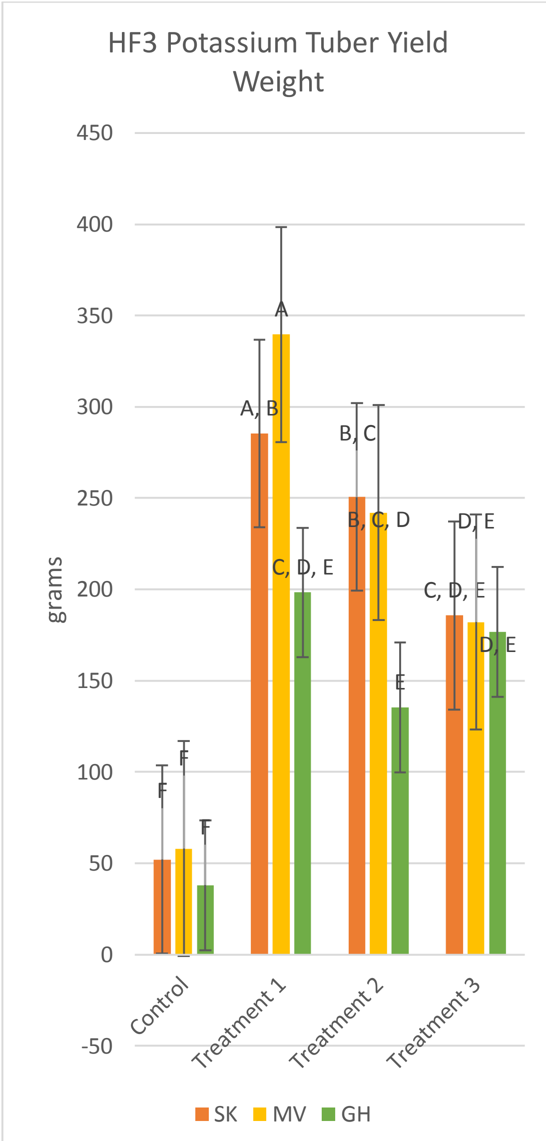


Figure 4 – The number of tubers produced by each flat at HF3. SK, MV, and GH are compared by treatments. HFE more than doubled the amount of tubers compared to CFR.	Figure 5 – The number of tubers produced by each flat at CFR. SK, MV, and GH are compared by treatments. SK fared on average the best with regards to production for all treatments.



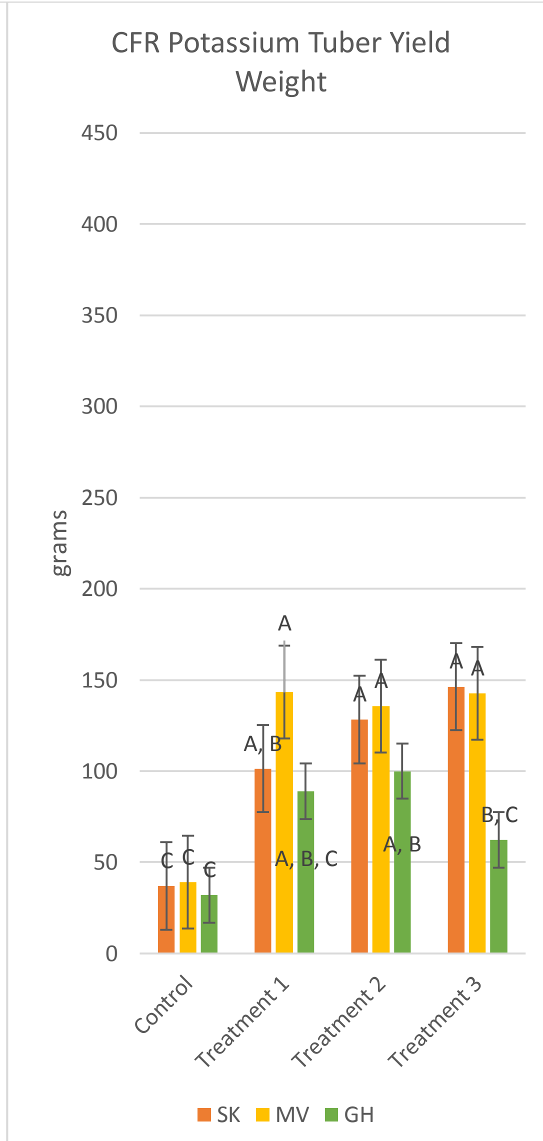
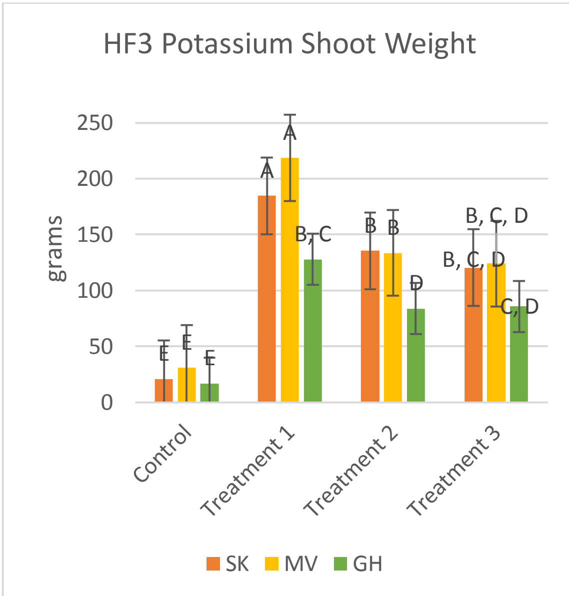


Figure 6 – The total weight of all tubers produced by each flat at HF3. SK, MV, and GH are compared by treatments. HF3 tubers weighed almost twice as much as CFR tubers.	Figure 7 – The total weight of all tubers produced by each flat at CFR. SK, MV, and GH are compared by treatments. Treatments did not seem to effect tuber yield weight.



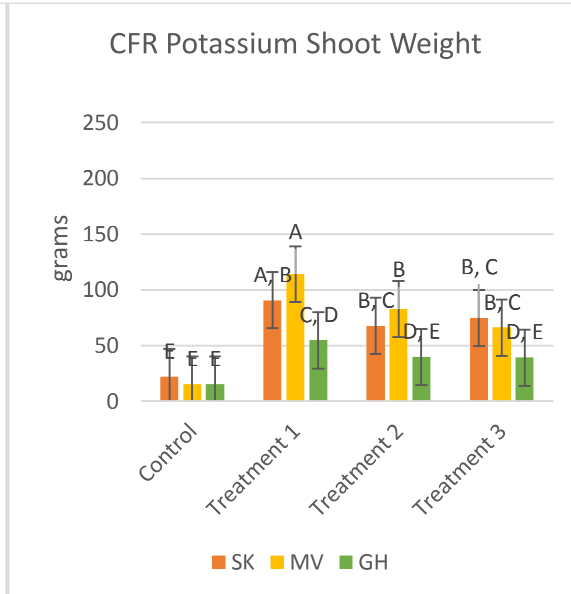
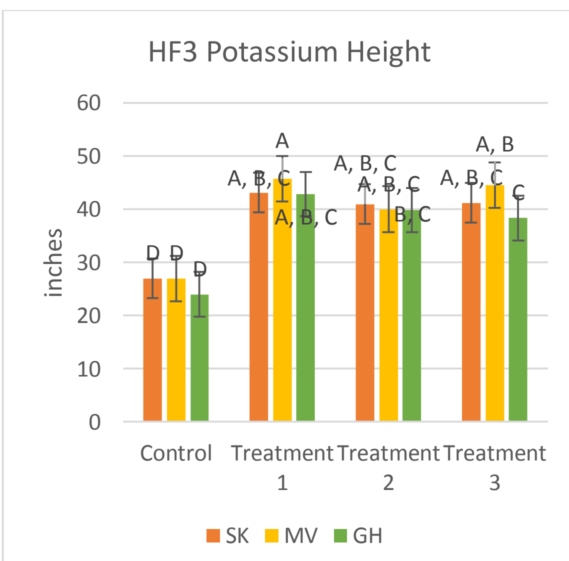


Figure 8 – The total weight of all shoots produced by each flat at HF3. SK, MV, and GH are compared by treatments.

Figure 9 – The total weight of all shoots produced by each flat at CFR. SK, MV, and GH are compared by treatments.



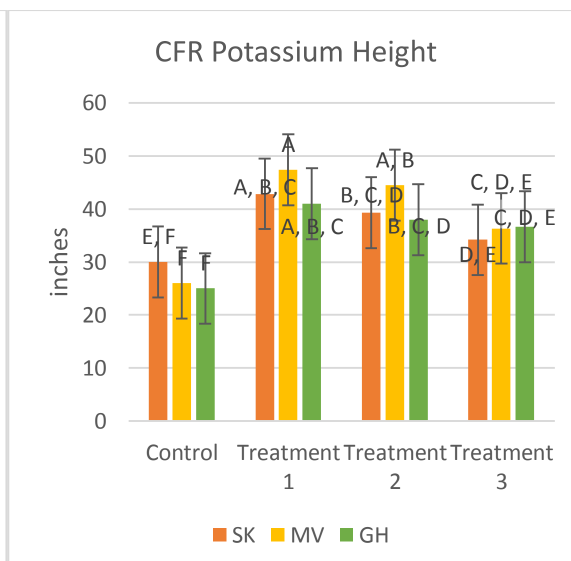
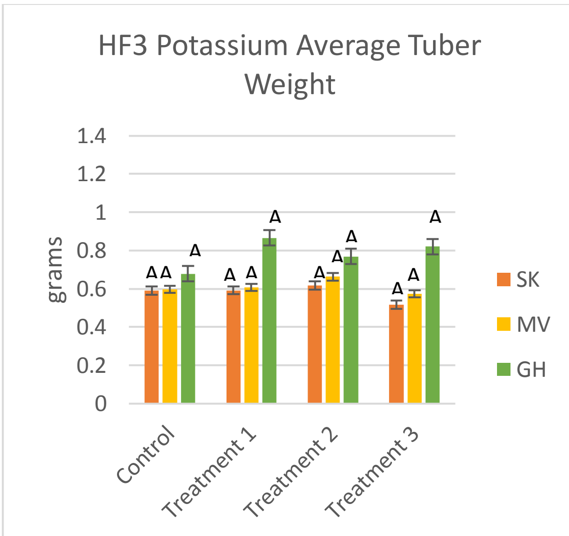


Figure 10 – The height of all shoots produced by each flat at HF3. SK, MV, and GH are compared by treatments.

Figure 11 – The height of all shoots produced by each flat at HF3. SK, MV, and GH are compared by treatments.



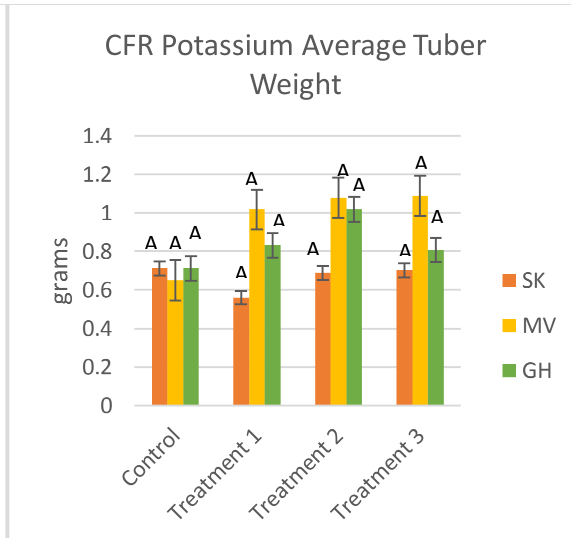
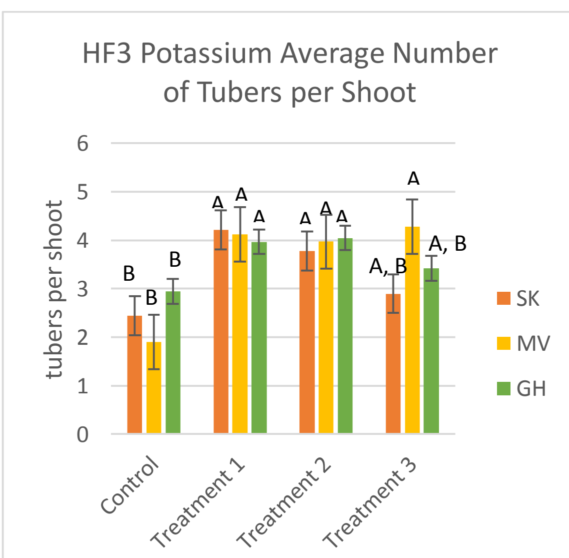


Figure 12 – The average weight of each individual tuber produced by each flat at HF3. SK, MV, and GH are compared by treatments. Tubers range in weight from about 0.5 grams to 0.9 grams.

Figure 13 – The average weight of each individual tuber produced by each flat at CFR. SK, MV, and GH are compared by treatments. . Tubers range in weight from about 0.5 grams to 1.1 grams.



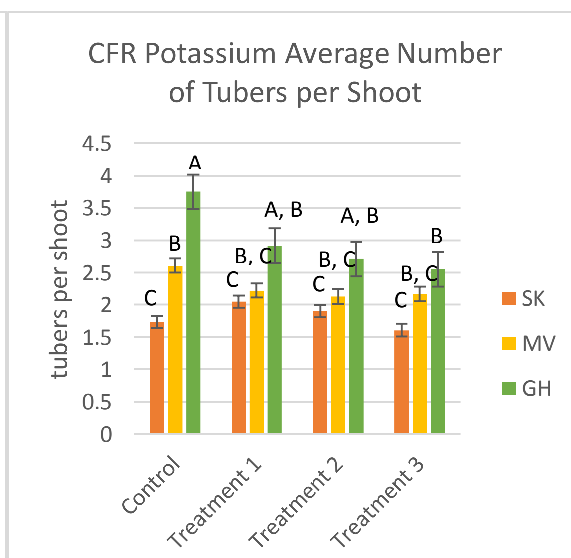


Figure 14 – The average number of tubers per shoot produced by each flat at HF3. SK, MV, and GH are compared by treatments.	Figure 15 – The average number of tubers per shoot produced by each flat at CFR. SK, MV, and GH are compared by treatments.

At HF3 and CFR, all treatments significantly increased the number of shoots, except at CFR, only GH did not produce significantly more shoots for all treatments. For number of tubers produced at HF3, Treatment 1 for all selections yielded the most tubers. Treatments 2 and 3 only yielded more tubers for SK and MV. At CFR, all treatments yielded more tubers for SK and MV only. GH was unaffected. At HF3, all treatments for all selections yielded more tuber weight than the control. For CFR, only SK and MV for all treatments yielded more tuber weight than the control. Treatments at HF3 yield more than double the weight of tubers, when compared with CFR. Shoot weight at HF3 was significantly higher for all treatments and selections, and was nearly double the weight of the shoots produced at CFR. Shoot weight at CFR was significantly more than the control for all selections of Treatment 1. Shoot weight was only higher for MV and SK at CFR for Treatments 2 and 3. Plant height was significantly higher than the control for all selections and treatments at HF3, and all treatments and selections at CFR, except for Treatment 3 of SK. Average tuber weight at HF3 was slightly higher in GH for all treatments than MV and SK. Average tuber weight at CFR was greater than HF3 for all treatments and selections. Average number of tubers per shoot were not affected by treatments.

Conclusion –

9, & 11). However, potassium increase had no effect on tuber yield weight (Figures 6 & 7). It was application of 20g of NPK (Treatment 1) that had the most dramatic effect on those two selections, especially to MV with regards to tuber weight, and shoot weight- MV was in the top tier. GH did not seem to do much better than the control except for treatment 2 on tuber weight, and all GH did better than the control with respect to height. It would seem that if a greenhouse farmer were going to grow for greatest plant production, in terms of tuber size and yield weight, it would be best to choose selections MV and SK. However, according to (Figure 13), GH and MV seem to produce on average the highest amount of large tubers. Depending on the market demand, GH and MV may be better to plant if larger tubers are desired. It would be better to plant SK for tuber processing, such as extraction of oils, or pulverization into flour, or squeezed for horchata juices. Figure 15 shows differences among selections with respect to number of tubers produced per shoot. There are no statistical differences either among treatments, or among selections with regards to number of tubers produced per shoot.

At HF3 –

All treatments performed greater than the control (Figures 2, 4, 6, 8, & 10). It should be taken into great consideration that selection MV with treatment 1 was in the statistical top-tier for every trait- tuber yield weight and number, shoot weight and number, and plant height. Other treatments did not do as well as Treatment 1 did. Since the hoop-house is closer to what is representative of in the field, farmers should take note that this is the selection and combination of fertilizer (20g NPK/m^2) that makes the plant perform in the best way. Other treatments were not consistently significant enough to have made any reasonable difference. Figure 14 shows that on average, fertilizer treatments stimulate more tubers per shoot. Figure 12 shows that on average, GH has a higher tuber weight- although this is not significant.

CFR and HF3 –

Treatments 2 and 3 generally did significantly better than the control, meaning that adding fertilizer of any type beyond the 10g of NPK/m^2 is beneficial to the plant, but not in any consistent manner, as treatments tended to have different results for each variable tested. However, for both places, MV with Treatment 1 was consistently in the top statistical tier for almost all variables. It is even more important to note that MV is the top for all variables at HF3, which has the closest conditions to what field conditions would be like. Although potassium did not have the tuber-enlarging effect that we were hoping for, we did discover that a balanced and high rate application of NPK significantly increases the production of selection MV. The other two selections GH and SK may be too closely-related, or respond to fertilizer in the same way to see too much of a difference between them. The ideal fertilizer condition for production is high, balanced fertilizer of NPK. The ideal environmental conditions for production indicate that a hoop-house is best for tuber production. Looking at Figures 6&7, the conditions of HF3 have double the amount of tubers compared to CFR. Looking at Figures 4&5, the number of tubers has doubled as well. Overall plant biomass has increased at HF3, which is directly correlated with tuber production (Figures 8&9). Perhaps this is because tuber production may be increased upon plant stress. Hoop house conditions are constantly changing with the outside weather, and this may be enough to stimulate the production of more tubers.

Chapter 3 – Characterization of Chufa: Iron Enrichment of Soils and Foliar Spray

Knowing that iron is an important plant and human nutrient, we wanted to see if we could increase the nutrition of the tubers, as well as gauge the effects of iron on plant productivity. Since iron is semi-soluble in soils, we wanted to test different soils for absorption and effects. For Iron Experiment 1, we chose to apply the iron at different times, to see the phenotypic effects. For another Iron Experiment 2, we chose professional growing medium (potting soil), clay-loam, and one commercial organic soil. For Iron Experiment 3, we delivered the iron as a foliar spray, to measure the effects on phenotype. For all iron experiments, we chose the selection, GH because it gave the most consistent results out of all the selections. (For literature on this matter, please refer to Chapter 1).

Objective – To determine the effects of timing iron applications on plant productivity. To determine the media which enhances iron effects on plant productivity. To determine if foliar application affects plant productivity.

Methods –

The iron over time experiment and iron growth media experiment (Iron experiment 1 and Iron Experiment 2) were planted during the week of July 6, 2014, and harvested 14 weeks later, on the week of October 12, 2014. The foliar application experiment (Iron Experiment 3) was planted during the week of July 5, 2015, and harvested 14 weeks later, on the week of October 11, 2015. All experiments were planted in a hoop-house style greenhouse at 67 Ryders Lane, East Brunswick, NJ, known as Horticulture Farm 3

(HF3). Only the GH selection was used. Selection germplasm was obtained from markets in Ghana. The pH was kept in the 6.4-7 range for all trials. Results were statistically analyzed using the "proc glm" command in the SAS software suite. Fisher's Least Significant Difference (LSD) model was applied to the data ($p<0.05$), and significance groups were generated and applied to the results. The highest significance level was marked as "A", with lower significance levels marked as "B", "C", and so on.

Iron Experiment 1 (Iron Over Time) –

Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with Pro-Mix® brand BX general-purpose professional growing medium soil. We refer to it as GreenHouse Mixture (GHM) soil. Three sprigs were planted per flat, and each of the controls received only 10g of NPK fertilizer. Iron was added to the experiments in the form of GroTech® Ironite soluble iron fertilizer in increasing concentrations- Treatment 1 = 10 days after 4 weeks after planting apply 10g of iron plus 10g of NPK/m^2. Treatment 2 = 20 days after 4 weeks after planting apply 10g of iron plus 10g of NPK/m^2, Treatment 3 = 30 days after 4 weeks after planting apply 10g of iron plus 10g of NPK/m^2. Treatment 4 = 40 days after 4 weeks after planting apply 10g of iron plus 10g of NPK/m^2. Treatment 5 = 50 days after 4 weeks after planting apply 10g of iron plus 10g of NPK/m^2. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber-formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed.

Rooty fibers were detached and discarded. Tuber weight is the weight of all tubers produced in a flat (tuber yield weight).

Iron Experiment 2 (Iron Growth Media) –

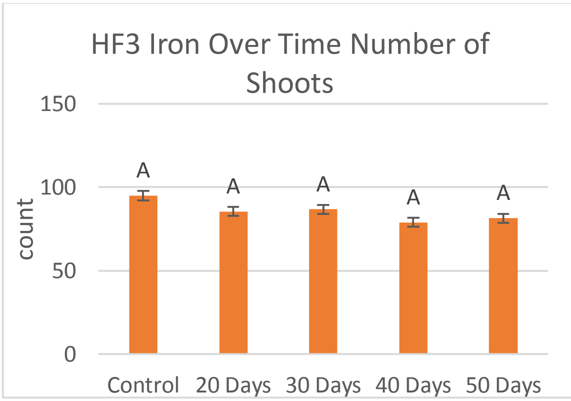
Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with either: Pro-Mix® brand BX general-purpose professional growing medium soil; we refer to it as GreenHouse Mixture (GHM) soil, Field soil (FS) collected from HF3 which is a clay-loam, or unammended MiracleGro® brand organic soil (OS). Three plants were planted per flat, and each of the controls received only 10g of NPK fertilizer. Only OS did not receive any fertilizer, as organic proponents claim that the soil is inherently as fertile as other soils, if not more-so. Iron was added to the experiments in the form of two different levels: $10g/m^2$ or $20g/m^2$ of GroTech® Ironite soluble iron fertilizer. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed. Rooty fibers were detached and discarded. Tuber weight is the weight of all tubers produced in a flat (tuber yield weight).

Iron Experiment 3 (Iron Foliar) –

Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with Pro-Mix® brand BX general-purpose professional growing medium soil. We refer to it as GreenHouse Mixture (GHM) soil. Three plants were planted per flat, and each of the controls received 17.5g of NPK and 15g of muriate of potash (MOP) fertilizer. Appended Iron was added to the experiments in the form of AquaIron™ DTPA chelated 10% soluble iron fertilizer. A concentration of 2ppm, 4ppm, and 8ppm was sprayed for treatments 1, 2, and 3, respectively. These treatments were done at 5 weeks, 7 weeks, and 9 weeks after planting. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber-formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed. Rooty fibers were detached and discarded. Tuber weight is the weight of all tubers produced in a flat (tuber yield weight).

Results –

Iron Experiment 1 (Iron Over Time Experiment) –



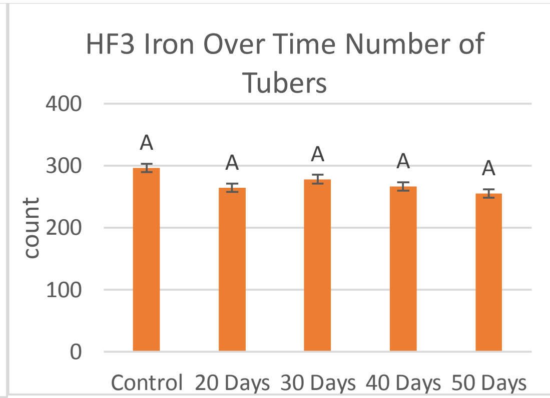
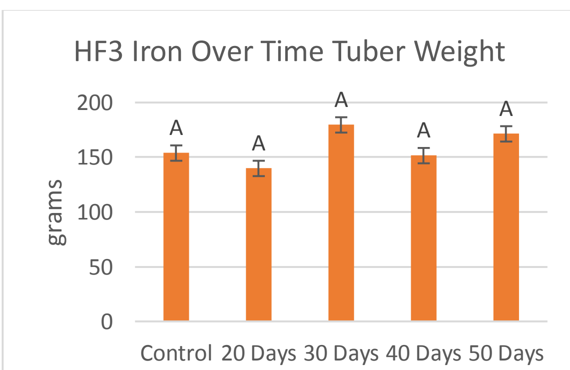


Figure 16 – Effect of iron application on the number of shoots produced by each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.

Figure 17 – Effect of iron application on the number of tubers produced by each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.



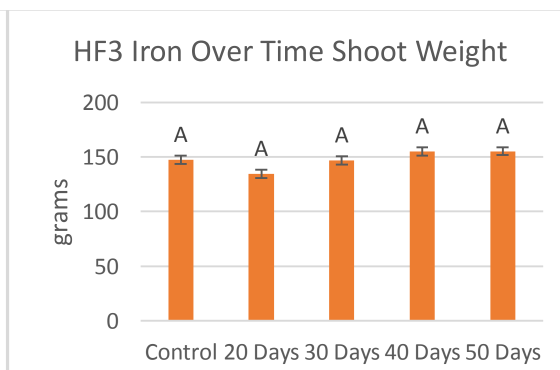
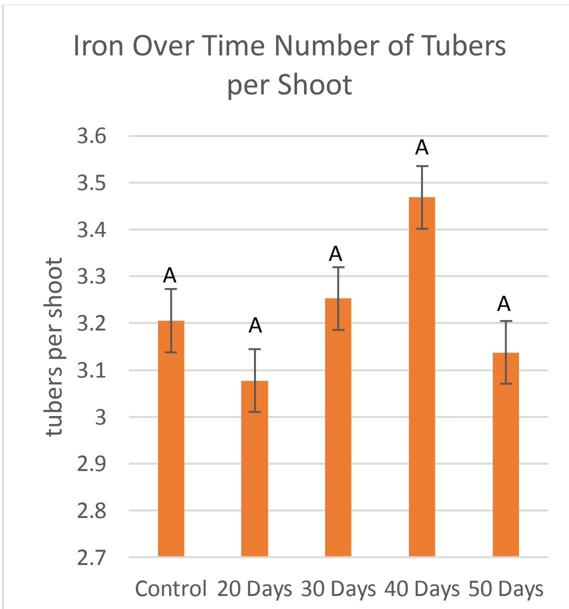


Figure 18 – Effect of iron application on the tuber yield weight of each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.

Figure 19 – Effect of iron application on the total shoot weight of each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.



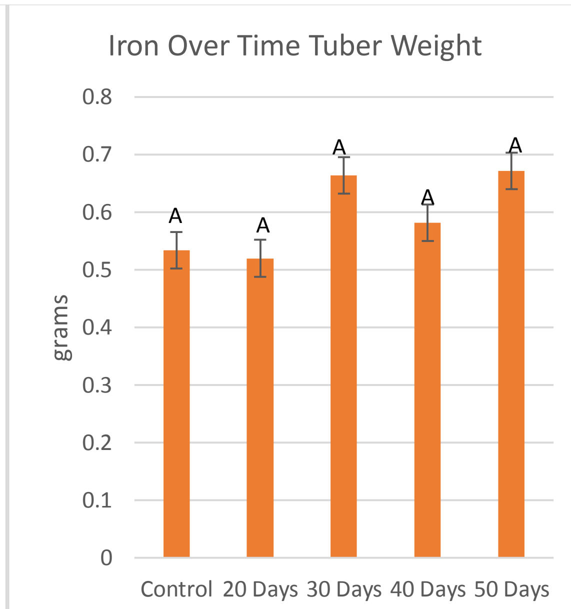
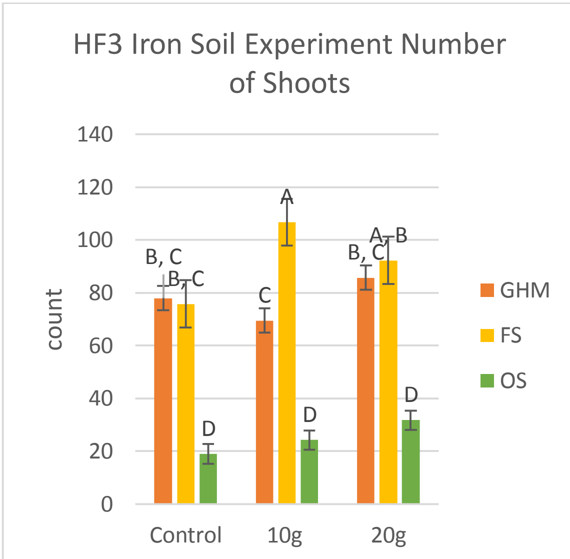


Figure 20 – Effect of iron application on the average number of tubers per shoot produced by each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.

Figure 21 – Effect of iron application on the average weight of individual tubers produced by each flat over time. Iron was applied at 4 weeks old (control), or 20 days after that, or 30, 40, or 50 days after that. 10g of iron was used. No significant difference observed.

No application of iron to the soil at any time had any effect on the physiology of the plant. No significant difference was observed.

Iron Experiment 2 (Iron Growth Media Experiment) –



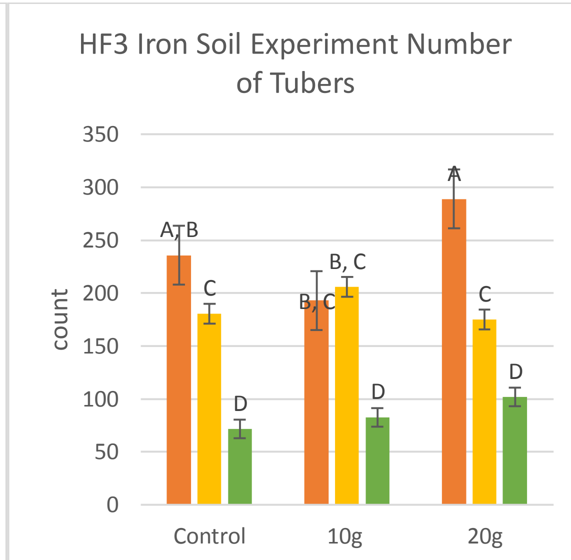
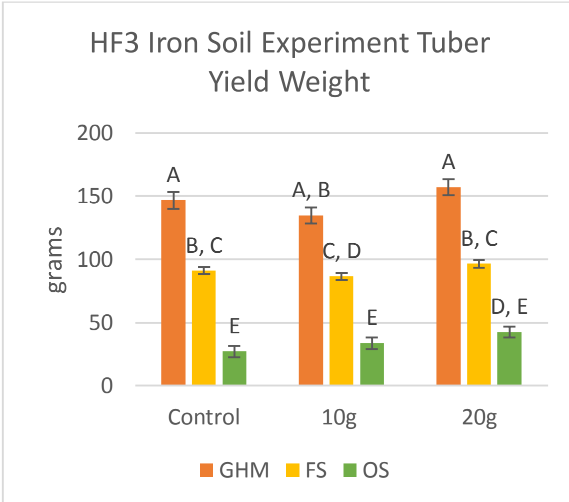


Figure 22 – Effect on number of shoots produced by applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.	Figure 23 – Effect on number of tubers produced by applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.



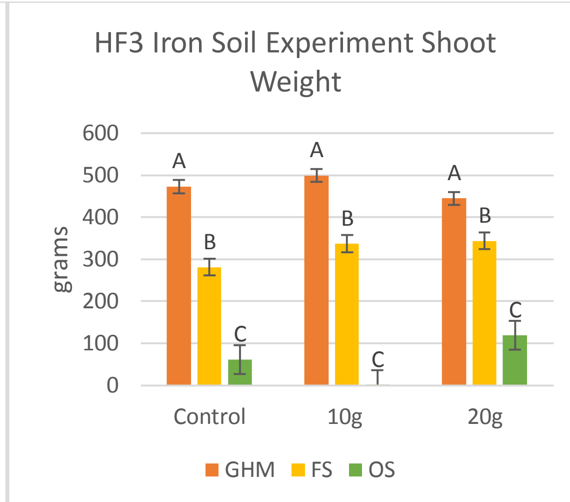
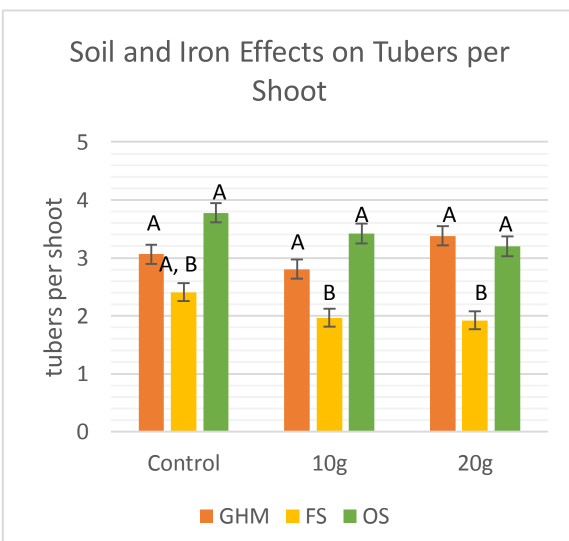


Figure 24 – Effect on tuber yield weight of applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.

Figure 25 – Effect on shoot weight by applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.



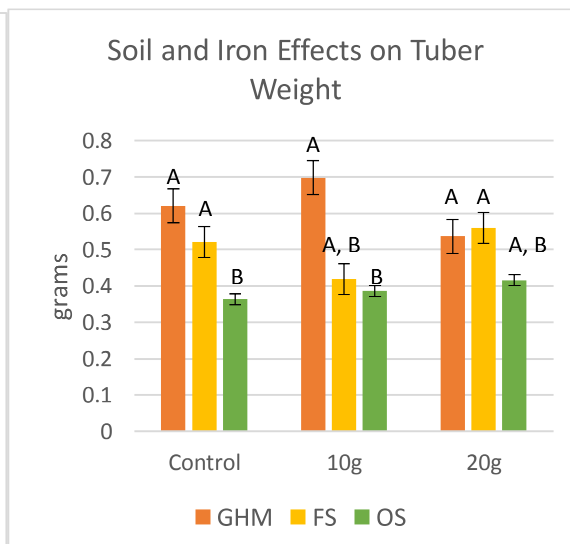
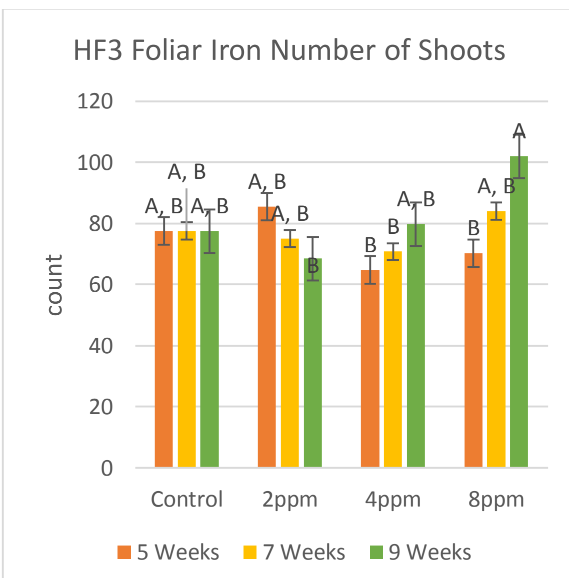
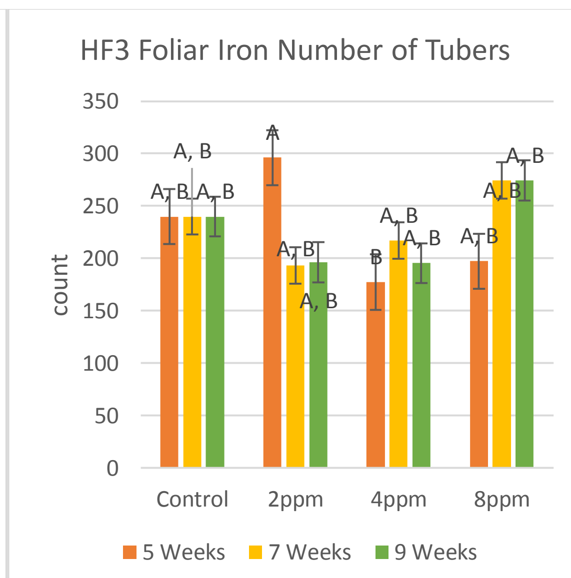


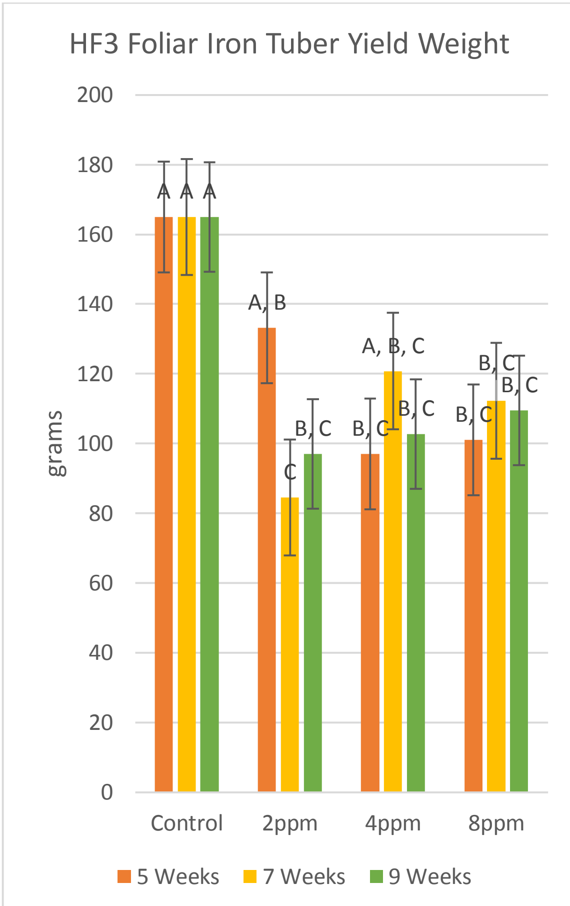
Figure 26 – Effect on average number of tubers per shoot produced by applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.	Figure 27 – Effect on individual weight of tubers produced by applying different amounts of iron in different soils. Fertilized GHM and FS out-performed unamended OS.

For all graphs and variables, plants grown in unamended OS fared significantly poorer than plants grown in GHM or FS. Iron application in any amount did not have an effect on plant physiology for any variable compared with the control. However, for tuber yield weight and shoot weight, GHM yielded significantly more weight compared to FS and OS. FS yielded significantly more weight compared to OS.

Iron Experiment 3 (Foliar Iron Experiment) –







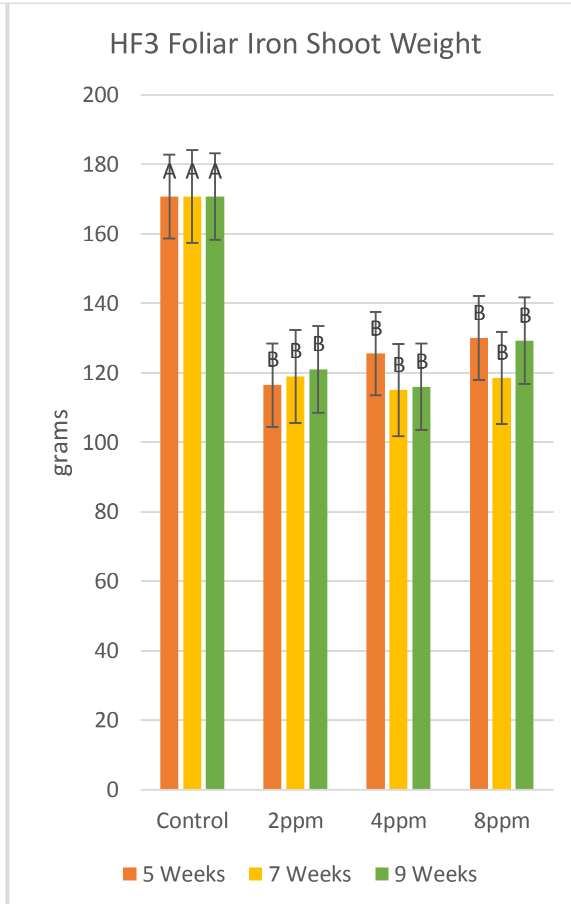
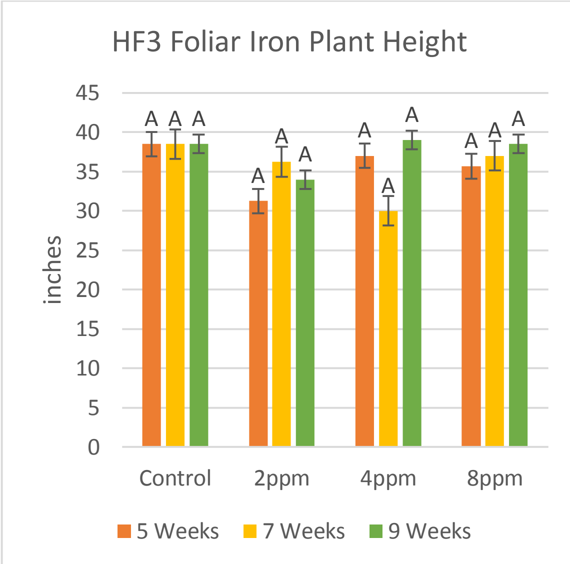


Figure 30 – Effect on tuber yield weight of applying different ppm of iron as a foliar spray on the leaves.

Figure 31 – Effect on shoot weight by applying different ppm of iron as a foliar spray on the leaves.



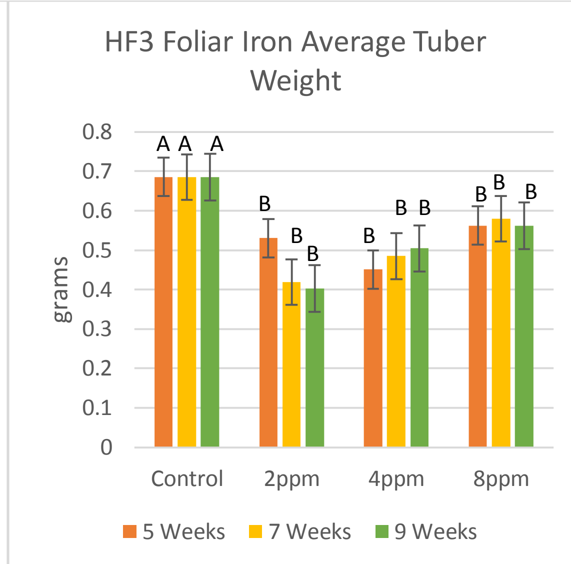


Figure 32 – Effect on plant height by applying different ppm of iron as a foliar spray on the leaves.

Figure 33 – Effect on average individual tuber weight by applying different ppm of iron as a foliar spray on the leaves.

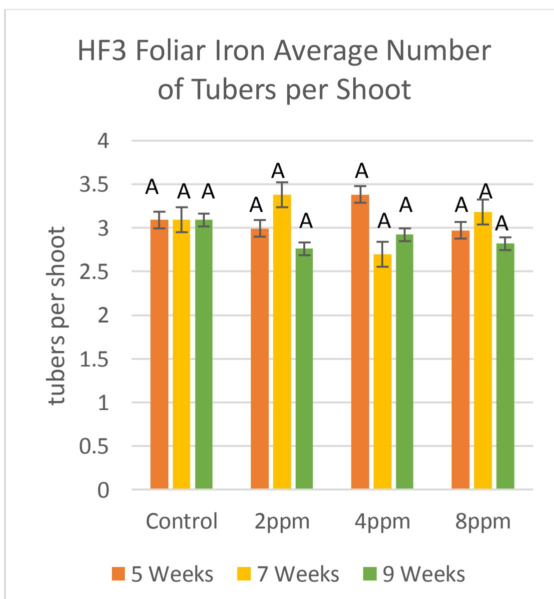


Figure 34 – Effect on average number of tubers per shoot produced by applying different ppm of iron as a foliar spray on the leaves.

Foliar spraying of chelated iron at any concentration had no effect on number of shoots or number of tubers produced. Tuber weight and shoot weight was depressed significantly at any application strength. Average tuber weight was also significantly decreased with foliar application.

Conclusion –

Iron Experiment 1 (Iron Over Time Experiment) –

Timing of soil iron application had no effect on any variable, as seen in Figures 16-21. Neither shoot height, shoot weight, tuber yield weight, nor tuber yield number was affected by iron application timing. No variable was significantly different from the control. This indicates that the timing of soil iron application has no effect on physiology.

Iron Experiment 2 (Iron Growth Media Experiment) –

As seen in Figures 22-27, all OS treatments and control performed significantly worse than other soils. This may be due to the fact that we did not add 10g of NPK to the soil. FS with 10g of iron added had significantly more shoots than its control, and more than any non-FS group (Figure 22). Overall, FS with 20g of iron added seemed to not have done as well as FS with 10g of iron added. With regards to number of tubers (Figure producing increased tuber weight. This particular set of results may be a quirk. With regards to tuber yield weight, GHM of all applications are all statistically in the highest set. In Figure 24, GHM yielded almost twice as many tubers as FS did. GHM control yielded significantly more than FS, which yielded significantly more than OS. This implies that for tuber yield weight, GHM is the way to go. For shoot weight (Figure 25), GHM significantly out-performed FS, which significantly out-performed OS. No differences were found based on the amount of the treatments. It would seem that iron application does not affect the productivity of the chufa. However, it may affect the amount of iron in the tubers.

Iron Experiment 3 (Iron Foliar Experiment) –

As seen in Figures 28-34, no level of any foliar spray had any effect on any variable, with the exception of tuber yield weight (Figure 30) and shoot weight (Figure 31). Foliar application of iron seems to be detrimental to tuber weight and shoot weight. If iron happened to be an effector, or some sort of growth suppressor, 2-8ppm is noticed by the plant. Compared to the effects of iron fortification of the soil, iron foliar spray seems to have a detrimental effect towards biomass and tuber yield. More studies need to be done to evaluate the effects of the foliar spray of other nutrients.

Chapter 4 – Characterization of Chufa: Effect of Irrigation on Chufa Productivity

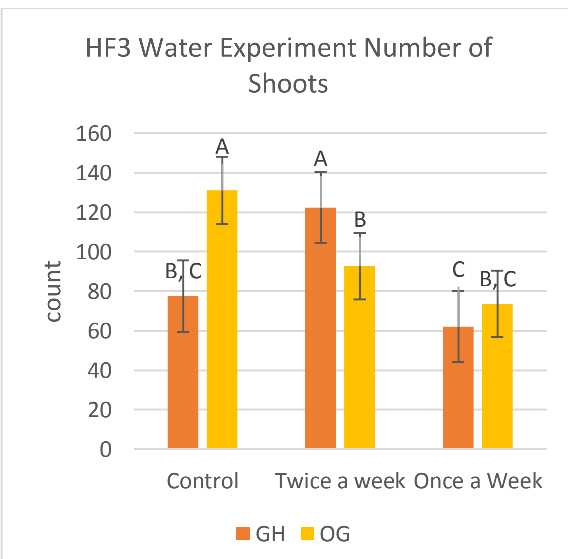
Chufa has been cultivated in North Africa as a food source for thousands of years, and has been cultivated often in moist, flooded soils. The wild relatives, "Yellow Nutsedge" and "Purple Nutsedge" (*Cyperus esculentus* L., and *Cyperus rotundus* L. respectively) are dispersed ubiquitously around the world in a variety of climates. These climates, range from arid to tropical. As such, we wanted to look at how water-frequency application affects plant productivity of our two best selections, OG and GH.

Objective – To determine the effects of restricted water applications on plant drought tolerance, productivity, and root density.

Methods –

Two experiments were planted during the week of July 6, 2015, and harvested 14 weeks later, on the week of October 12, 2015. The experiment was planted in a hoop-house style greenhouse at 67 Ryders Lane, East Brunswick, NJ, known as Horticulture Farm 3 (HF3). Two different selections of Chufa were used- OG and GH. Selection germplasm was obtained from markets in Ghana and Nigeria. Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with Pro-Mix® brand BX general-purpose professional growing medium soil. We refer to it as GreenHouse Mixture (GHM) soil. Three plants were planted per flat, and each of the flats received 15g of NPK fertilizer and 15g of muriate of potash (MOP). The control received 2 liters of water every day. Trial 1 received 2 Liters of water 2 times a week per flat. Trial 2 received 2 Liters of water once a week per flat. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber-formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed. Rooty fibers were detached and discarded. Results were statistically analyzed using the “proc glm” command in the SAS software suite. Fisher’s Least Significant Difference (LSD) model was applied to the data ($p<0.05$), and significance groups were generated and applied to the results. The highest significance level was marked as “A”, with lower significance levels marked as “B”, “C”, and so on.

Results –



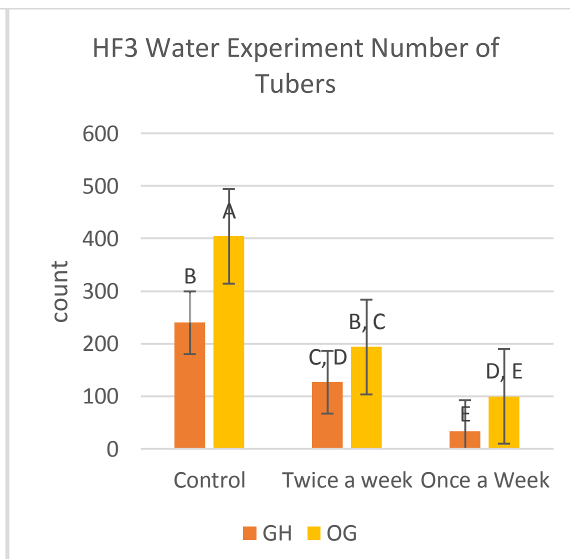
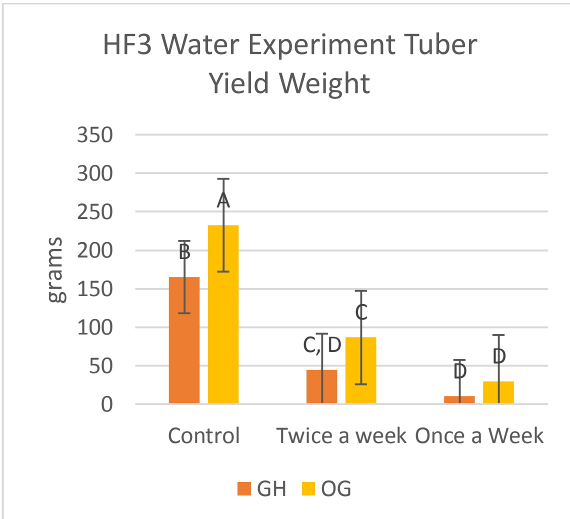


Figure 35 – Effect of irrigation on number of shoots produced. More water significantly improves plant productivity.

Figure 36 – Effect of irrigation on number of tubers produced. More water significantly improves plant productivity.



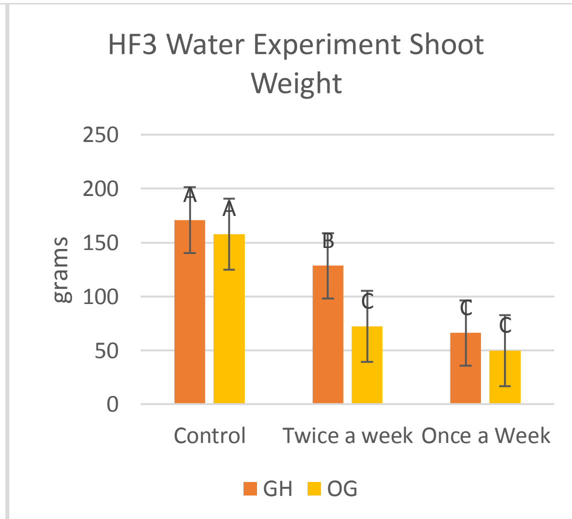
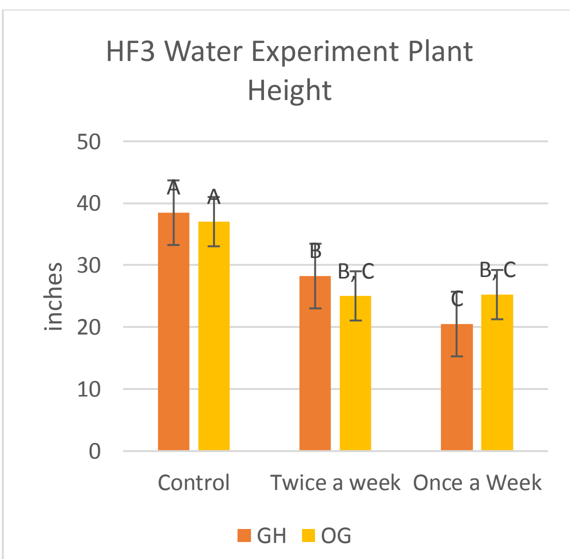


Figure 37 – Effect of irrigation on tuber yield weight. More water significantly improves plant productivity.

Figure 38 – Effect of irrigation on shoot weight. More water significantly improves plant productivity.



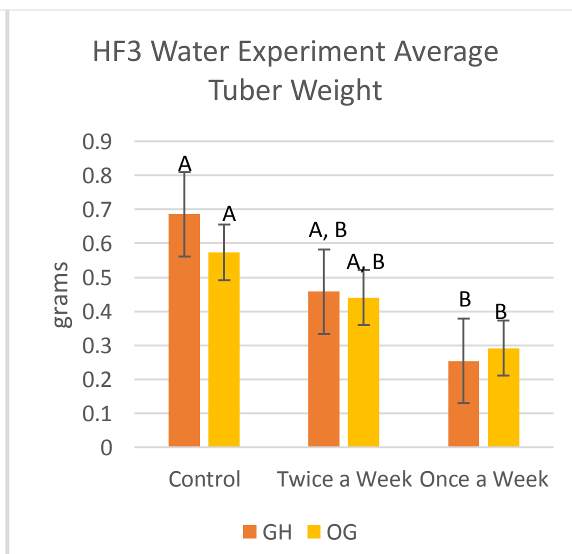


Figure 39 – Effect of irrigation on plant height. More water significantly improves plant productivity.

Figure 40 – Effect of irrigation on average individual tuber weight. More water significantly improves plant productivity.

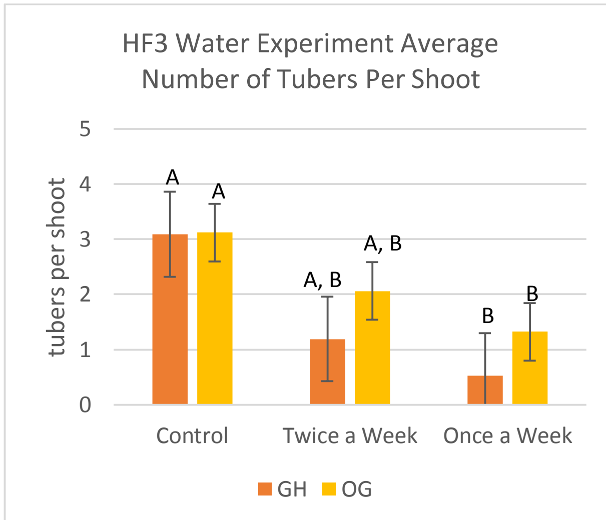
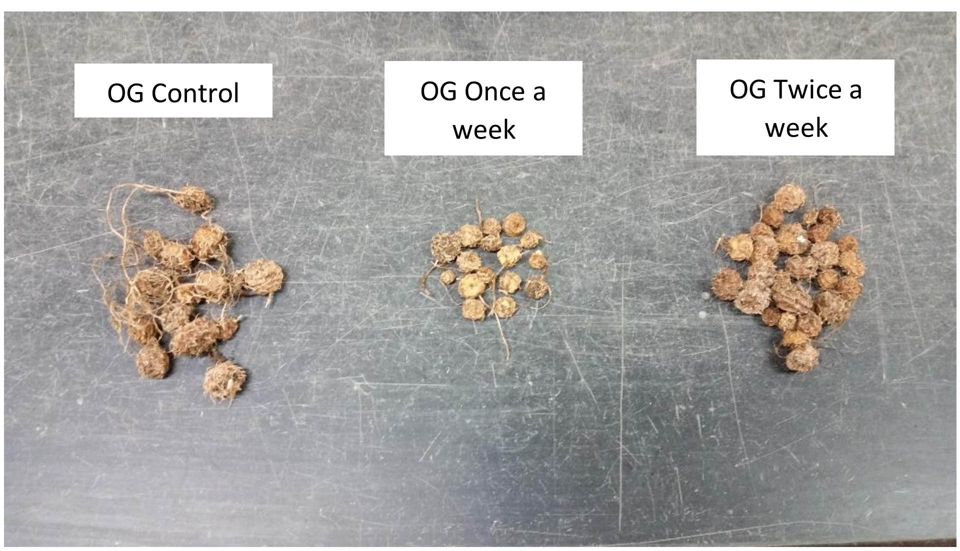


Figure 41 – Effect of irrigation on average number of tubers per shoot produced. More water significantly improves plant productivity.



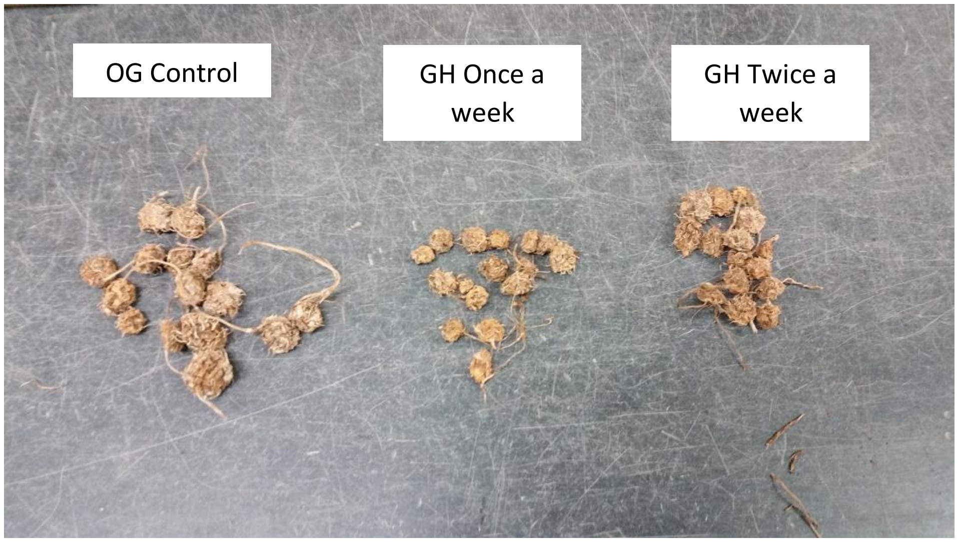


Figure 43 – Irrigation has a visible effect on root density in GH. More frequent irrigation produce more visible roots when harvested.

Irrigating every day significantly increases all variables compared to restricted irrigation. Tuber and shoot weight of Chufa irrigated every day is double that of Chufa irrigated less than that. Plant height, tuber weight, and average number of tubers per shoot is also increased with increased irrigation. As seen in Figures 42 & 43, increased irrigation also increases root density around the harvested tubers.

Conclusion –

As seen in (Figure 35), for number of shoots, OG that was irrigated every day and GH that was watered every 2 days performed significantly better than any other treatment. With regard to the number of tubers produced (Figure 36), OG watered every day did significantly better than any other selection or treatment. GH irrigated every day came in second with regards to tuber production. Both selections irrigated once a week fared the poorest. Tuber yield weight, shoot weight, and height (Figures 37, 38, & 39) were highly affected by water application amount. OG fared the best with irrigating every day, followed by GH watered every day. Average tuber weight, and average number of tubers per shoot decreased with decreasing water (Figures 40 & 41). As seen in Figures 42 & 43, root density around the tubers increases with increased irrigation for both selections of Chufa. As the water application decreases, so too does productivity. This shows that Chufa productivity (and root density) is directly related to water application frequency. With Chufa being such a water-thirsty crop, this may have implications on where it can be planted. For example, in areas with water shortages, it would not be wise to plant this crop. However, in frequently-flooded areas, this crop would be ideal. Chufa has been historically planted along the Nile River in Egypt. The Nile is famous for its predictable, yearly floods, which have brought fertile sediments from the highlands of Ethiopia. Given the results of the Potassium Experiment (See Chapter 2), Chufa does indeed perform better with higher levels of balanced nutrients. Recreating these ideal conditions may be the key to success with this crop.

Chapter 5 – Characterization of Chufa: Soil-Type Effects on Chufa Productivity

Ecological and Bioremedial Roles

The genus *Cyperus* is already ubiquitous across the globe, and well-suited to the NYC environment, as well as the coastal environment in which it would be planted. Of course, in this case, Chufa would be used for its bioaccumulative and organic-compound-detoxifying abilities (Bamishaiye, 2011). The strong rhizomatous roots are also ideal in preventing soil erosion (Ghosh, 2003). They are often the first colonizers of barren and disturbed land, and keep the soil stable enough for ecological succession of larger plants to occur.

There have been some efforts to use Chufa as a bioremediator, and as a biostabilizer (McPhearson, Hamstead, & Kremer, 2014). A case study shows that New York City (NYC) has some of the most polluted waterways and runoff in the country, and that projects that involve remediation via greenspace save the city up to $1.5 billion annually (McPhearson, Hamstead, & Kremer, 2014). Using Chufa along with other bioremedial plants would be an excellent way to expand upon an already beneficial program. The increase of greenery would contribute to erosion control, beautification, civic pride, reduction of CO_2, absorption of runoff and rain water, and create jobs.

Cyperus is also a known food source of many wildlife native to NY and NJ. Hunters have been known to plant Chufa in order to attract wild turkey (Bamishaiye, 2011). In fact, Chufa has been used to save the declining turkey population all across the eastern United States (Hammond, 2014). Chufa is a preferred food for wild turkeys, being so nutritious, and state governments have planted acres of Chufa to successfully rescue the turkey population. The tubers are important for other waterfowl as well. They are an essential part of the greater Northeastern US ecosystem.

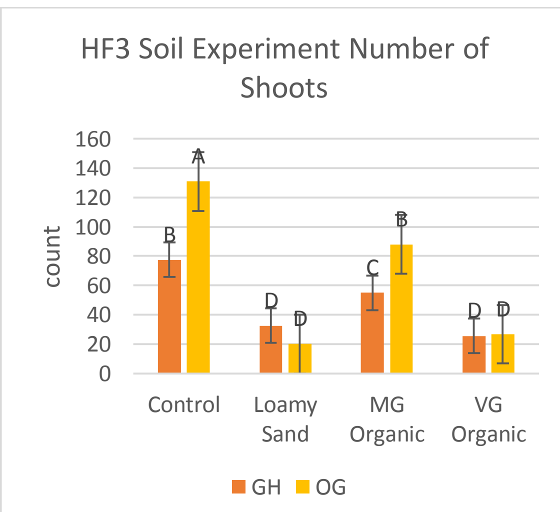
We thought it important to test in which soil Chufa does the best. We had predicted that lighter, sandy soils would increase tuber production and size, while reducing the root density of the tubers. As such, we chose 4 different soil conditions to test- professional growing medium, loamy sand, and two commercial organic soils made by different companies. We also chose two of our best selections to perform in this trial, to measure differences between selections, to find the best conditions for productivity for each selection. (For literature on this matter, please refer to Chapter 1).

Objective – To determine the effects of different soil types on plant productivity.

Methods –

Chufa was propagated by taking the stalk a semi-mature plant with some of its roots intact, trimming it down to about 4 inches, and planting it into a standard greenhouse flat filled with either: Pro-Mix® brand BX general-purpose professional growing medium soil; we refer to it as GreenHouse Mixture (GHM) soil, field soil collected from Rutgers Agricultural Extension at Adelphia, NJ (a loamy-sand, LS), Vigoro brand Organic Soil (VG) or organically-fertilized MiracleGro® brand organic soil (MG). Three plants were planted per flat, and each of the non-organic soils received 15g of NPK fertilizer and 15g of muriate of potash (MOP). MG and VG received the recommended amount of 2oz. per flat of MiracleGro® dry organic fertilizer. Plants were given their fertilizer once, about 4 weeks after planting, right before tuber-formation. Plants were grown until 14 weeks had passed, then harvested, and data for shoot weight, shoot number, tuber weight, and tuber number was collected. Tubers were washed, then dried for two days, then weighed. Rooty fibers were detached and discarded. Tuber weight is the weight of all tubers produced in a flat (tuber yield weight). Results were statistically analyzed using the "proc glm" command in the SAS software suite. Fisher's Least Significant Difference (LSD) model was applied to the data ($p<0.05$), and significance groups were generated and applied to the results. The highest significance level was marked as "A", with lower significance levels marked as "B", "C", and so on.

Results –



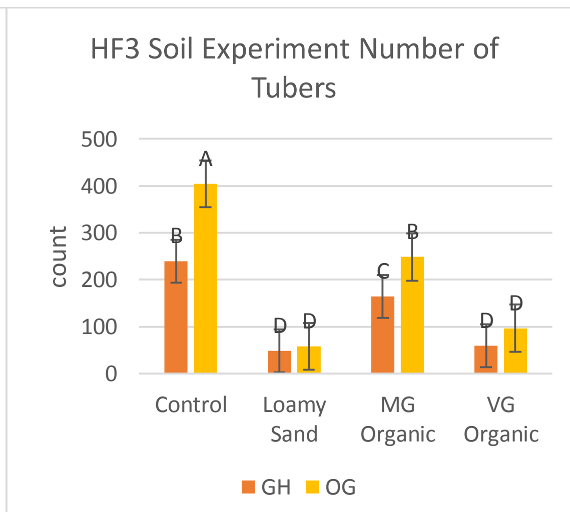
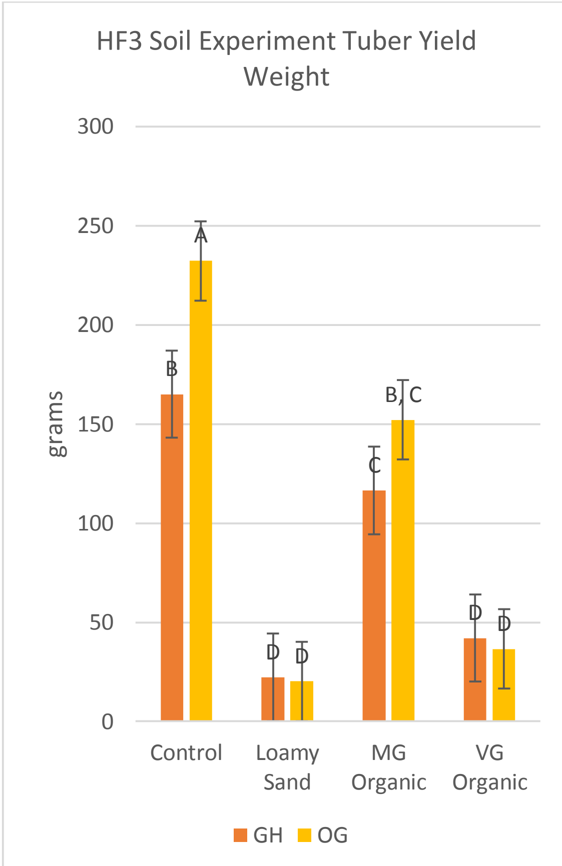


Figure 44 – Effect of soil type on number of shoots produced. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.

Figure 45 – Effect of soil type on number of tubers produced. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.



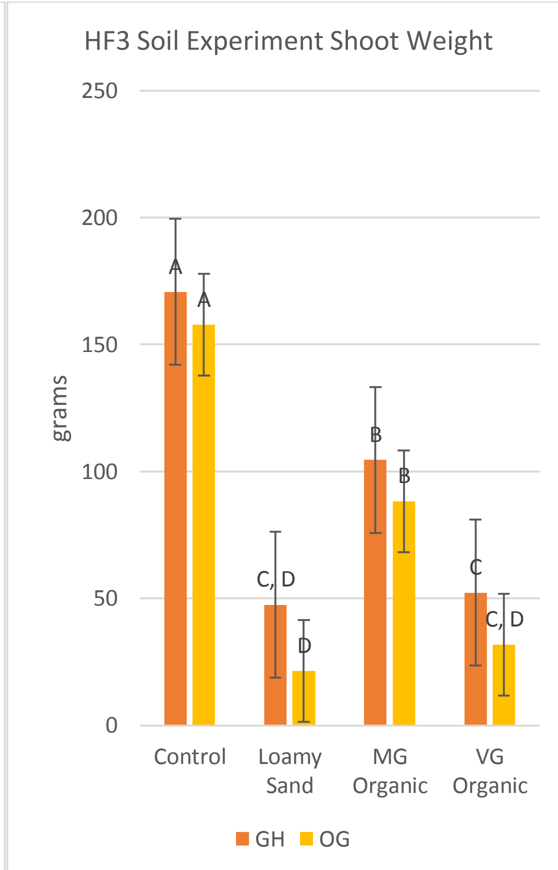
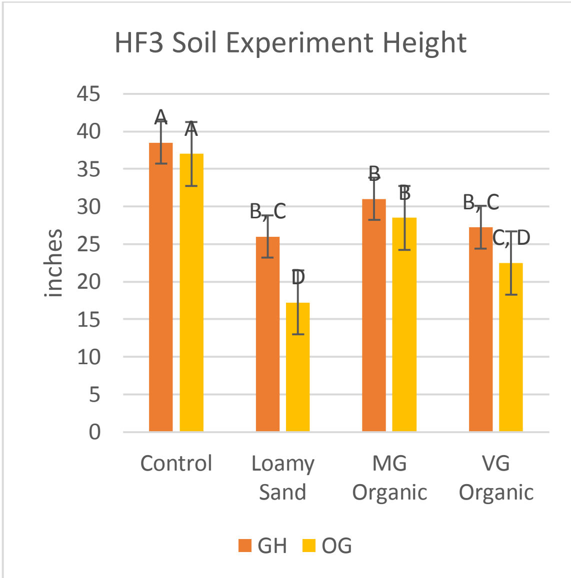


Figure 46 – Effect of soil type on tuber yield weight. Besides the control, MG Organic soil yielded the most tubers. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.

Figure 47 – Effect of soil type on shoot weight. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.



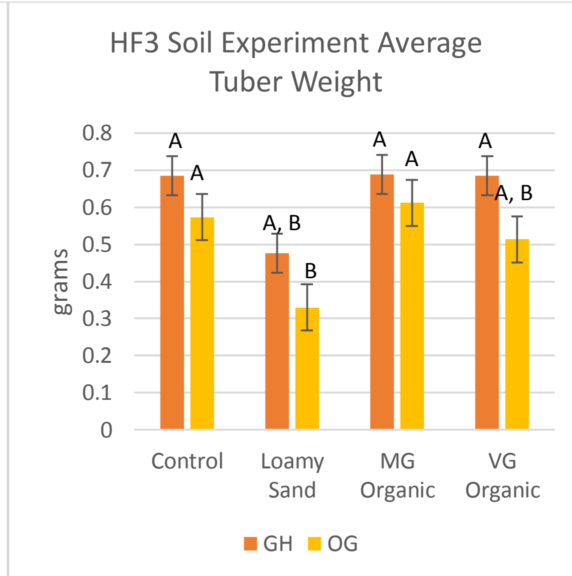


Figure 48 – Effect of soil type on plant height. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.

Figure 49 – Effect of soil type on average individual tuber weight. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.

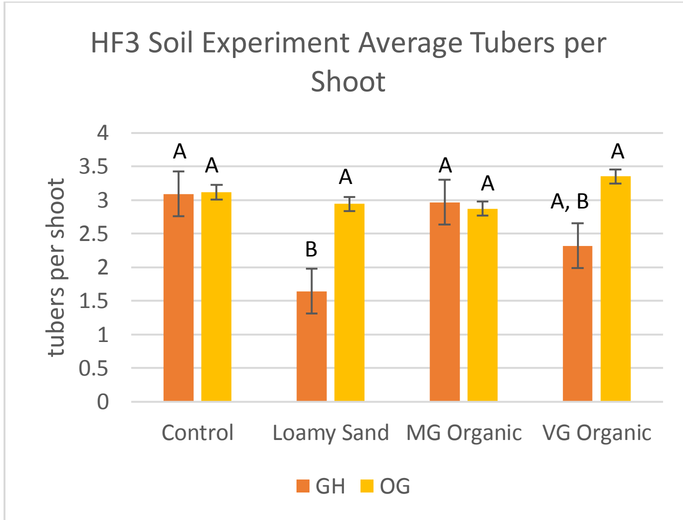


Figure 50 – Effect of soil type on average number of tubers per shoot produced. Loamy-sand produced less-root-dense tubers, whereas GHM control and MG Organic produced root-dense tubers. The control and MG organic soil performed the best overall.

For number of shoots, number of tubers, tuber yield weight, and shoot weight, Chufa grown in the professional growth medium (GHM) produced significantly the most, followed closely by Chufa grown in MG. Chufa grown in LS or VG fared the poorest, producing less than half the number of shoots, number of tubers, tuber yield weight, and shoot weight. However, Chufa grown in LS produced clean tubers with almost no roots attached. Chufa grown in LS also happened to be the easiest to harvest, requiring a fraction of the time compared to harvesting from other soils.

<u>Conclusion –</u>

As seen in Figures 44, 45, & 46, OG in GHM did the best at making many shoots and tubers, as well as tuber yield weight. This was followed by OG in MG, and GH in GHM and MG. Both selections in LS and VG made the fewest number of shoots. For shoot weight (Figure 47), Both GH and OG did the best in GHM, followed by both selections in MG. VG and LS did significantly poorer. Average number of tubers per shoot was unaffected by soil (figure 50). With respect to plant height (Figure 48), both selections grown in GHM did significantly better than the others. It would seem that the ideal soils for Chufa productivity are high in organic matter, but highly digested organic matter. The VG soil seemed to have been almost a wood-chip mulch, rather than a finer soil, like the other soils. MG seemed highly-digested as an organic soil, and was finer in particulates, and comparable to GHM in consistency. The VG soil dried out quite quickly, and perhaps the poorness of performance in VG may be due to the fact that the low-water was hampering the productivity of the plant (See Chapter 4).

Although Chufa in LS was not very productive, it should be noted that the tubers were almost bare and root-free, compared to tubers produced in other soils. Tuber individual size was also, on average, significantly smaller than in other soils (Figure 49). LS was also the easiest soil in which to harvest the tubers. This may be advantageous, as chufa is almost impossible to harvest in heavier soils, such as clay. We had noticed that last year, planting the Iron-Soil experiment (See Chapter 3), the field soil was a clay-loam, and was extremely difficult to harvest tubers from. We also had thought that the tuber size was inversely proportional to soil-heaviness. This has proven to be not true. In-fact, these results suggest that Chufa is a plant that prefers organically-rich, but moderate-to-light soils. A future experiment should involve adjusting the concentrations of sand and loam to find the ideal soil that makes harvesting easy, and

Chufa create the least amount of roots. Because Chufa performs so well with high amounts of fertilizer (See Chapter 2), we highly recommend that farmers amend their soils with as much organic matter and fertilizer as possible.

Chapter 6 – Current Status and Future Applications

Although Chufa is not popular in the Western world yet, it certainly is growing as a commercial food product. In Wholefoods stores, Tigernut Horchata is sold on the same shelf as cold-pressed juices. Tiger Nuts are sold in small bags and with trail mix to the Paleo-diet market. However, two major limitations to this crop achieving national attention are that Chufa is incredibly difficult to harvest, and the machinery in Spain that does harvest Chufa is not for sale, and that Chufa is considered to be a noxious weed in most farms.

We believe that the consumer markets will dictate demand of this crop, and if the price is right, farmers' opinions will be swayed about the status of this plant. Also, if the harvesting equipment is developed, then Chufa can finally be advertised to the national markets, and break through as a health food. With such benefits as all 20 amino acids, high mineral and caloric content, versatile use, its usage history in Egypt, and a flavor like coconuts and almonds, it is likely that Chufa will be a hit with the American public.

Because Chufa is considered a "weed", and weeds seem to fare better than actual crops themselves, it is highly productive to begin with. It has virtually no pests, save for Billbugs. This makes it attractive as a crop. However, with the water demands of the crop, it is likely to be planted along riversides, or in easily-flooded fields where other crops do not like to grow. This will help with land requirements, and potentially opens up floodplains and riverbanks as potential cultivatable land.

Ecological and Urban Farming Applications

Urban growth and dynamics have called for a need to address the growing issue of food sustainability- in growing, processing, transport, and consumption. In addition, the recent concerns in global climate change, economic stability, and food health concerns have made Urban Agriculture (UA) an issue of great importance (Specht, et al., 2014). UA has been an activity of leisure, but has resurged as an important factor in urban sustainability and economic resilience (McClintock, 2010). With unavoidable increasing urbanization (Nations, 2004), the need for new ways of bringing local, fresh, affordable produce becomes priority to the economic sustainability and food supply of cities (Elijah's Promise, 2015; Brock, 2008). With the ever-growing “Go Green” movement, public demand for fresh produce, and the recent economic downturn, UA has been implemented as a means of food security, job creation, creates leisure, and a sink for urban waste (McClintock, 2010; Specht, et al., 2014; Mougeot, 2005; UNDP, 1996). UA has also been used as city beautification and as community gardens, creating green spaces and leisure spaces in cities, where there were none before. Community gardens have been used in urban restoration projects and community strengthening projects in the Bronx, with many health benefits to the volunteers besides having access to healthy local food (Ottmann, 2012). Food shelters such as Elijah’s Promise in New Brunswick, NJ have benefitted from food donations from local community gardens and home-growers in the City of New Brunswick. Elijah’s Promise has trained many volunteers in how to grow their own food, where food comes from, giving education and job-training for those interested, as well as encouraging community engagement and helping the city's hungry (Matacera, 2015).

Community gardens can help needy families save money on food every year, as well as be profitable for others. Community gardens' productivity can be measured as economic value per square foot. Average NYC community gardens can raise $1-10 per square foot, but have seen as high as $40 per square foot (Saldivar-Tanaka & Krasny, 2004; Vitiello & Nairn, 2009; Gittleman, Jordan, & Brelsford, 2012). A small garden can raise about $50,000 in fruit and vegetable sales, while larger gardens can generate even more revenue.

We have found that chufa planted in about 2 square feet flats have yielded on average 500 tubers in outdoor conditions with average tuber size about 0.8g, which is about 400g of tubers. Market retail value of Chufa tubers ranges from about $0.68-1.10 or more per ounce (TNUSA, 2015), or $0.0239 per gram. That makes 400g of tubers worth about $9.59-15.52. This is about $4.79-7.76 per square foot, which is competitive with high-value crops such as tomatoes and beans (Saldivar-Tanaka & Krasny, 2004). The advantage of Chufa over beans and tomatoes, is that it requires little to no maintenance. Beans need to be culled into trellises, and tomatoes need to be steaked individually, costing time and money. Chufa is a "set it and forget it" type of crop, with the obvious exception of irrigation, weeding, fertilizer applications, and other general crop maintenance. Chufa is also advantageous to plant because it is relatively pathogen-free. No insect damage except for Billbug has been observed during experimental trials at field experiments at Rutgers Horticultural Farm 3, where it is planted and maintained.

Organizing with "Organic Gemini Tigernuts" company in Brooklyn, NY and the community gardens in Brooklyn, we can outreach to the local community and encourage the growth of the Chufa and promote its benefits. The harvest from the community gardens can be sold to Organic Gemini Tigernuts, benefitting the community with revenue, greenspace, fresh produce, and helping the local chufa/tigernut business. With a possible future Rutgers-patented harvesting machine, Rutgers could stand to benefit from the revenue generated from sales, rental, and use of these machines that we have developed. The ecological and economic potential of Chufa to be used in this way is great, and increasing as demand for fresh, local produce and local greenspace increases.

Given that the Chufa market in the Americas is just opening up, that leaves few competitors and an unsaturated, open market. Because Chufa/Tiger nuts are healthy, tasty, and versatile in use, they can be incorporated into a wide variety of products and industries including the baking industry, the gluten-free market, and most importantly, the health food industry. We fully believe that more exploration can be done with different fertilizers and different soils and their effects on tuber production and plant productivity.

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