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Improving the Nutrient Content of Crops through Mutualism

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

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and Renée Crown University Honors
May 2016

Honors Capstone Project in Biology

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Abstract

Agriculturists continually look for ways to improve the nutrient content of crops without decreasing yield or economic benefits. Mutualistic relationships have the potential to enhance the nutrient content without sacrificing the production needs of the farmer. Mutualisms occur when two or more species interact and both members of the association benefit. An exceedingly important and often overlooked mutualism is the one formed between arbuscular mycorrhizal fungi (AMF) and plants. This interaction has been shown to be a critical component of most ecosystems, yet our understanding of these relationships is still limited. We know that in exchange for photosynthetically derived carbon, AMF help to increase plant nutrient uptake. However, the potential of AMF to improve the crop nutrient content relative to human health is relatively unstudied. Optimal levels of mutualistic activity could increase efficiency in agriculture, and these advancements would improve the economic and environmental impacts of agriculture.

To assess the benefits of AMF on crop nutritional value, I designed a greenhouse experiment that tested the effect of AMF inoculation on carrots planted in nutrient deficient sand. I used two AMF species, *Rhizophagus clarus* and *Rhizophagus intraradices* and compared the effect of carrots grown with these AMF species individually, both together, and without AMF. I examined above- and belowground biomass as well as the levels of beta-carotene and a suite of minerals. The results showed that carrots grown with both AMF species had increased biomass, aluminum, phosphorous, and zinc levels and showed trends of increased beta-carotene. This suggests that AMF application in agriculture could increase the availability of nutrient dense crops and help sustain the global food supply.

Executive Summary

Arbuscular mycorrhizal fungi (AMF) sounds rather complicated, but in reality it is a simple soil organism that associates with over 90% of all plants. It is one of thousands of microscopic soil species that make up the underground ecology of plants. AMF have characteristics that make them a good candidate for agricultural application because they form a mutualism with plants. Mutualisms are a type of symbiotic relationship where both members of the relationship benefit. In the case of AMF, the fungi provide the plant with nutrients found in hard to reach spaces of the soil and the plant provides the fungi with carbohydrates from photosynthesis. The exchange occurs in fungal structures termed arbuscules that develop once the fungi establish inside the plant root cells. The fungus grows thread-like hyphae that extend from the plant root into inaccessible soil spaces and sequesters important nutrients needed for plant growth and maintenance. Research shows that the AMF-plant mutualism increases plant nitrogen and phosphorous levels. However, there are limited studies evaluating if the mutualism increases the crop nutrient content relevant to *human* health.

Increasing the nutrient content in crops is of topical importance to the current global supply food crisis. The world population is expected to reach 9 billion by the year 2050. In order to keep up with the growing population size, current agriculture yields need to increase by at least 70%. In addition, climate trends predict that the atmospheric level of carbon dioxide will continue to increase. As CO_2 increases, the mineral content of crops decreases. Therefore, it is critical to find a way to increase the quality of the crops. AMF provide an economically and environmentally sustainable approach to agriculture.

The goal of my research project was to assess the benefit of the mutualism between AMF and crop plants with respect to the nutritional content relevant to human health. I believe the

application of my research extends beyond the scientific community to affect the global community. In order to test my hypothesis, I tested the effect of two AMF species, *R. intraradices* and *R. clarus* on the growth and nutrient content of carrots. I set up four different treatments in a greenhouse experiment: two single species AMF treatments (*R. intraradices* or *R. clarus*), one treatment with both species, and a control treatment where no AMF species were applied. Carrots were grown in sterile sand with limited water application to simulate nutrient deficient soils. The carrots were harvested after 20 weeks, and biomass and nutrient content was determined.

The results showed the carrots grown with AMF had significantly higher aboveground and belowground biomass. An increase in biomass is enticing for farmers who desire ways to maximize production. In addition, the AMF increased aluminum, phosphorous, and zinc levels and decreased molybdenum and sodium levels. The results also indicated a trend of increased beta-carotene with AMF application. Beta-carotene is important for our body's production of vitamin A. Vitamin A deficiencies rank among the most common and debilitating deficiencies in the world. Deficiencies are most common in areas of poor soil quality. If AMF can increase the levels of beta-carotene in sterile sand, it is indicative that AMF application could allow people living in areas of poor soil to receive more beta-carotene and therefore decrease instances of vitamin A deficiency. Zinc deficiencies are also common and receiving adequate amounts of this mineral is necessary for proper nutrition. Zinc is an essential nutrient for DNA repair and immune response. If AMF can increase the content of these important nutrients in crops, agriculture application could reduce deficiencies around the globe.

The results suggest AMF application in agriculture can aid in supplying the growing world population with nutrient rich crops. This method is better than the current methods used to increase crop yields to feed the world. Right now, the solutions are actually making the situation worse. We use chemical filled pesticides and fertilizers that leak into our environment cause more damage. The application of synthetic fertilizers results in nitrogen runoff in waterways and contamination of drinking water. If ingested, the nitrogen reduces our ability to carry oxygen. Moreover, the release of oxidized nitrates from the application of fertilizer also contributes to the formation of smog, greenhouse gasses, and destruction of the ozone layer. AMF application is clearly a more sustainable solution to sustaining the food supply by increasing the quality of a single serving of produce. In addition, past studies reveal that AMF increase the integrity and fertility of soil. AMF have the capacity to neutralize harmful soil toxins by storing them in structures called vesicles. This mechanism highlights their potential use in urban agriculture. City pollutants make soils unusable, AMF have the capacity to make the soil suitable for food production.

The results of my study combined with previous research on the benefit of AMF in agriculture suggest application of these underground fungi would enhance the quality of crops and the security of the global food supply.

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Preface

Under the mentorship of Professor Kari Segraves, I have been a working on my research project titled, “Evaluating the effect of arbusular mycorrhiza fungi on agriculture, for the past three years”. It has been an amazing experience studying and learning from Professor Segraves and the other members of the “Segraves lab”. The lab focuses on ecological relationships and has opened my eyes to important symbioses in our environment and their community importance. With the freedom to design my own research project, I decided to take the knowledge gained from the Segraves lab on mutualisms and apply it to broader topics. My research combines academic disciplines to challenge a question: How can we environmentally and economically sustain the global food demand? My research has evolved around discovering if a particular type of fungi, Arbuscular Mycorrhizal Fungi (AMF), could improve the nutritional content of a crop and aid in solving global concerns.

In addition to working in the greenhouse and laboratory, I presented my research artistically in a gallery setting (see appendix). This challenged me to visually and orally present my project in a way that transcended to a broader audience. Ultimately, this experience taught me the importance of explaining scientific research to the general community in order to have a wider impact. In addition to learning to better translate the information, this opportunity also connected me with students and community members who became interested in applying AMF to the Syracuse community. Over the next year, I intend to work with a small group of motivated people to apply my research findings to the urban gardens in Syracuse.

Acknowledgements

There is not enough chocolate or Cheetos in the world to purchase to thank Drs. Kari Segraves and Dave Althoff for their support and guidance. They have opened up their laboratory to share valuable knowledge and techniques. They have gone beyond and dedicated themselves to the growth and development of our entire lab group. They offer important life lessons that extend beyond scientific research. It is inspiring to see their passion for research and their selfless pursuit to improve ecological understanding. Thank you Kari and Dave for being the best role models.

The Renée Crown Honor's program is full of outstanding faculty. I am thankful for the services and opportunities provided through the college. Without the provided funding, my project would have not been financially feasible.

Thank you to the biology department for allowing me to access expensive equipment. Dr. Frank was instrumental in demonstrating how to use a Wiley mill in my study. I am also thankful for the beautiful, pristine upkeep of the Syracuse Greenhouse.

Thank you to Sigma Xi for a research grant, and for providing funding to many important projects that are advancing society.

Thank you to Professor Ed Morris for his excitement in my project. Ed challenged me to take my research outside of the science framework and reach a larger population. He continues to brainstorm ideas for future agriculture and sustainability and I am excited to work with him to apply my research to the Syracuse community.

Thank you to everyone in the Segraves Laboratory who have offered their constant support, editing advice, and friendship: Laura Porturas, Thomas Anneberg, Pristine Mei, Maizy Ludden, Haley Plasman, Alice Fox, and Shengpei Wang.

Finally, thank you to my family for encouraging me through this project. Thank you to my sisters, Shannon and Mary, for their patience and excitement when listening to me talk about some crazy sounding fungi :)

I could not have done this without the support and love of so many individuals. I am blessed to be a part of this esteemed institution and to be surrounded by so many incredible people.

Thank you!!!

Advice to Future Honors Students

It’s never too early to start working on your capstone. Be passionate about your research. Surround yourself with hardworking and inspiring individuals. Don’t forget to smile:)

Chapter 1

Introduction

The ability to increase our future global food supply relies on innovative agricultural techniques to increase crop yield and nutrient content. These techniques are needed because the vulnerability of the food system is increasing due to pressures from an increasing world population and detrimental climate change effects. For instance, world population is expected to reach nine billion by the year 2050 (Fig. 1). In order to sustain this growth alone, the UN Food and Agriculture Organization predicts agricultural yields must increase by at least seventy percent (O'Donoghue et al. 2011).

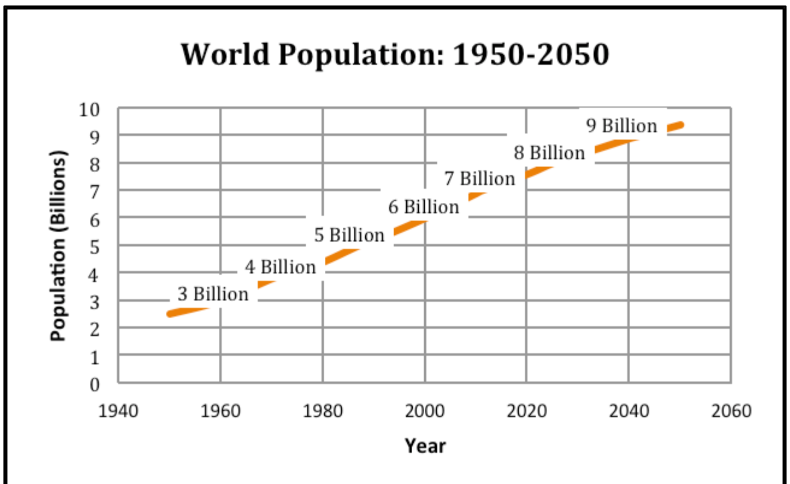

Figure 1: Population growth trend according to the U.S. Census Bureau.

Moreover, the situation is further exacerbated by current climate trends. As atmospheric carbon dioxide levels rise, crop plants have decreased concentrations of minerals and nutrients such as iron, zinc, and protein (Loladze 2002). As the nutrient content of crops decreases, larger production quantities are required to meet human macronutrient and micronutrient needs. Even without taking into consideration the expected population and climate trends, there are currently more than nine million people experiencing hunger due to insufficient access to nutrient dense food (Gardner & Halweil 2000). Studies reveal that large areas of hunger are in locations with the world's poorest soil conditions (e.g., Henao & Baanante 2006). Because poor soil conditions inhibit plant growth, nutrient content, and maturity, we must find a way to increase crop yield and quality to help abate world hunger.

One relatively understudied solution to abating the global food crisis is integrating underground species interactions and community ecology to create a more sustainable agricultural system. Plants interact with underground microorganisms such as rhizobium bacteria and fungi that transform organic soil matter into plant nutrients and aid in carbon and nitrogen acquisition (Bender 2015; Lambers et al. 2009). The diversity of interactions between soil organisms and plant roots can ultimately determine the viability of plants and may increase overall soil fertility. These types of symbiotic relationships have been shown to increase the productivity of agricultural systems (Medina et al. 2010). In particular, mutualisms, interactions where both members of the association benefit, have the potential to enhance the nutrient content of crops without sacrificing production yields or product quality. Therefore, an emphasis on community ecology and underground soil species interaction is necessary to sustain the global food supply.

In particular, a key mutualism that could increase the yield and quality of crops is the interaction between plants and arbuscular mycorrhizal fungi (AMF). The mutualism between AMF and plants is a critical component of most ecosystems. Indeed, at least 90 percent of plant families form relationships with at least one type of mycorrhiza (Plenchettee el al. 2005). In exchange for photosynthetically derived carbon, AMF help increase plant nutrient uptake. AMF can also neutralize toxic soil chemicals, increase resistance to disease and harsh environmental conditions, and help plants defend pathogens and predators (Karban 1989). Although there has been extensive research on the ability of AMF to forage for nutrients for plant maintenance, the potential of AMF to improve the nutrient value relative to *human* health is relatively understudied.

One reason AMF could increase the nutrient quality of crops is their ability to colonize the roots of plants and establish external structures that enhance the uptake of nutrients (Clark and Zeto 2000; Karaginnidis et al., 2007; Leigh et al., 2009; Veresoglou et al., 2010). Once the AMF enter the root cortical cells, they extend thread-like hyphae through the soil to maximize soil exploration and increase nutrient availability to the plant. The hyphae mobilize hard to reach nutrients and exchange them with the host plant through structures called arbuscules. The majority of AMF studies recognize AMF success in increasing plant phosphorous and nitrogen uptake (Leight et al., 2009; Johansen 1996), and evidence also supports increase of other nutrients including, zinc (Seres et al., 2006), copper (Toler et al., 2005), potassium, and iron (Cavagnaro 2008, Kim et al., 2010). These characteristics suggest the potential for this mutualism to increase crop yield in poor soil conditions. Indeed, in one of the few studies to address this, Hart and Forsythe et al. (2012) showed *Allium*, a highly mycorrhizal dependent plant, to have a strong positive nutrient response to AMF inoculation. This study represented an initial survey for the effect of AMF on nutrients important for human health. These results suggest that, from agricultural perspective, AMF application could increase nutrient crop quality and in turn, increase global food security.

I examined this possibility by testing how AMF affect nutrient content and yield of a common agricultural crop. In this study, I observed the effect of two AMF species, *Rhizophagus clarus* and *R. intraradices*, on carrots (*Daucus carota*). I chose to work with carrots because of their high mycorrhizal dependency due to their short root hairs that are inefficient at acquiring nutrients in depleted soils (Dechassa et al., 2003). In addition, the success of AMF and carrot symbiosis has been highlighted by a recent study that showed positive physiological and morphological root changes and increased carrot yield when carrots were inoculated with AMF (Affokpon et al. 2011). Carrots also have been shown to respond to a number of fungi species. I used the AMF species, *R. clarus* and *R. intraradices,* because they have a high success rate of infection in many key agricultural crops including carrots (Paradi 2003). Also, arbuscular colonization appears to peak earlier in these two species than many other species of AMF, which was conducive to the timeframe of my study (INVAM-WVU). Here, I tested if the successful mutualism of carrots with *R. intraradices* and *R. clarus* produces increased nutrient content benefits relevant to human consumption. This study addresses whether AMF application in agriculture could increase crop quality and suggest a viable solution to help meet future food demands.

Chapter 2

Materials and Methods

Creating bulk AMF

In order to perform the study, I first created bulk inoculum. Inoculum consisted of three main sources: AMF spores, infected root pieces, and hyphae. The AMF inoculum was applied to the soil in order to allow it to colonize roots (Smith and Reed 2008). I obtained single-species inocula of *R. clarus* and *R. intraradices* from the University of West Virginia International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM-WVU). These pure inocula were grown on the "trap" plant *Zea mays* to create a large volume of bulk inoculum for each AMF species. I chose corn as a trap plant because it is a C4 plant with high ATP requirements that would optimize sporulation and mycotrophic dependency. Prior to growing corn and AMF, I sterilized pots, sand, soil, and seeds to eliminate the chance of bacterial or fungal contamination. The sand and soil were autoclaved twice for 45 min with a 24 h rest period to ensure that any heat-stimulated microbes were killed. The corn seeds were sterilized by soaking in 10% bleach for 10 min and then washed five times with autoclaved water. The sterilized seeds were subsequently soaked overnight in sterile water to enhance germination.

Seeds were planted five per pot to maximize root development. I covered the bottom holes of each pot with tape and poked smaller holes in the tape for controlled water drainage. The pots were assembled by adding approximately a 7 cm section of autoclaved sand, followed by a mixture of 25 ml of AMF mixed and 400 ml of autoclaved soil, finished with a layer of sand with five corn seeds evenly sown (Fig. 2). This approach maximized root contact with the fungi as the corn roots penetrated through the soil. The plants were given low phosphorus fertilizer to increase the plant reliance and dependency on AMF (Plenchette et al. 2005). To make a 1 gallon supply of fertilizer 3g of Peters Professional 15-0-15 Peat Lite Dark Weather Feed Fertilizer, 0.45g $MgSO_4$, 0.75 mL of Scotts Miracle-gro 4-12-4, Quick Start Liquid Plant Food, and 3785mL of H_2O was mixed for a minimum of ten min. Each corn pot received 240 mL each treatment once a week (.19 g of Peters, 28.1mg $MgSO_4$, 0.0469mL of Quick Start, and 236.6mL of H_2O). The corn was watered every third day. Colonization was confirmed using staining techniques (described below) and the association with AMF formed within fifty days. Watering was stopped one week prior to inoculum harvest to increase spore production (INVAM-WVU) then aboveground biomass was removed and the roots and soil were chopped. The inoculum for each species was mixed well and stored at 4°C.

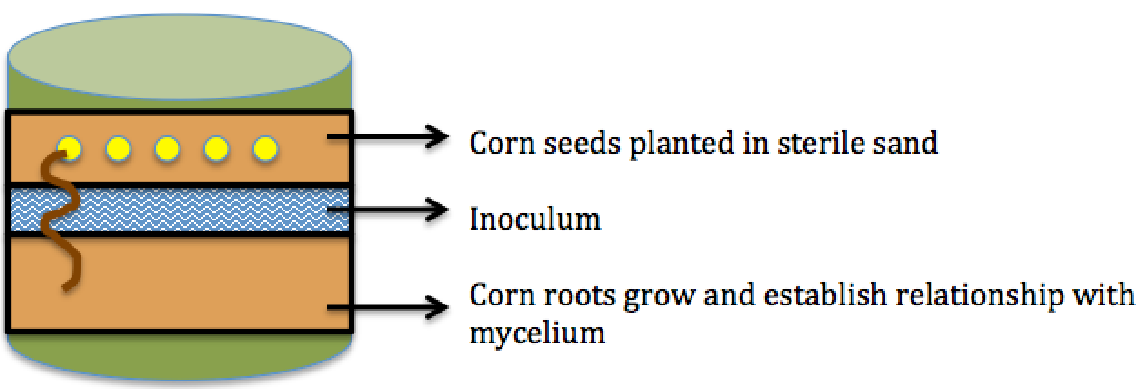

Figure 2: Experimental design for corn inoculation. The tan layers represent the sterile sand, the blue middle layer represents the added AMF inoculum, the yellow circles represent the corn seeds and the brown root represents sprouting from the seed and root establishment.

Carrot Study

The bulk supply of inoculum was used to test the effect of AMF on carrot nutrient levels and biomass. I used Gurney's scarlet nantes carrots because of their short root hairs and mycorrhizal dependency. Carrots were germinated in sterilized sand and transplanted once they had grown to about 2.5 cm. I transplanted the seedlings into sterilized 10 cm wide pots lined with punctured foil for controlled drainage and filled with autoclaved sand. Individual carrots were placed in a depression containing 25 mL of bulk inoculum or sterile sand. I used autoclaved sand to simulate low quality soil. There were four treatments with 20 replicates each. The experimental design consisted of 20 carrots grown with *R. intraradices*, 20 with *R. clarus*, 20 with both AMF species, and 20 controls lacking AMF inoculation. Following the addition of inoculum, the pots were topped with sterilized sand. Plants were placed in the Syracuse University Life Sciences greenhouse, arranged in a block design on the bench to minimize position effects in the greenhouse (Figure 3). Plants were given an initial 80 mL of the low phosphorous fertilizer treatment mentioned above to ensure successful transplantation. Plants were watered every third day, but not fertilized again.

Green House Carrot Pot Arrangement

A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A
C	D	A	B	C	D	A	B	C	D	A	B				
D	A	B	C	D	A	B	C	D	A	B	C				
A	B	C	D	A	B	C	D	A	B	C	D				
B	C	D	A	B	C	D	A	B	C	D	A				

Figure 3: Pot arrangement in the greenhouse. A=R. intraradices, B=R. intraradices and R. clarus, C=R. clarus, D=Control; no AMF.

Staining technique to test for infection

To ensure the roots were infected with the fungi treatment, I used the methods of Brundett and Bougher (1996). In brief, to clear the roots, they were autoclaved for 15 min in 10% KOH. The roots were rinsed with water before being transferred to a chlorazol black E staining solution. The roots were stained by autoclaving for 15 min and leaving them at room temperature for 24 h. Roots were scanned at 100X magnification and the presence of arbuscules and vesicle structures indicated positive infection.

Carrot Harvest

Carrots were allowed to grow for 20 weeks and then were harvested. To harvest, the pot contents were emptied onto a tray to remove the sand. The aboveground biomass (stem and leaves) was removed and placed individually into paper bags. The belowground tissue (carrot) was removed and placed into a separate paper bag. Fine root hairs were collected into 1.5 mL microcentrifuge tubes filled with 70% ethanol. Above- and below-ground tissues were dried in a 70 ˚C drying oven overnight. Once dry, aboveground and belowground biomass was assessed using a Mettler Toledo balance and a Mettler AC 100 balance.

Nutrient Testing

Carrots were finely ground using a Wiley mill fitted with a 40 mesh sieve. Samples were sent to the Cornell Nutrient Analysis Laboratory to test for aluminum, boron, calcium, copper, magnesium, molybdenum, manganese, sodium, phosphorous, and zinc using a dry ash extraction method. The samples were also tested for total carbon and nitrogen using combustion analysis. A small subset of samples were sent to Microbac Laboratory headquarters in Pittsburgh, Pennsylvania to be tested for beta-carotene levels.

Statistics

ANOVA was used to examine whether aboveground and belowground biomass differed between the treatments. A one-way ANOVA was also used to test for significant differences in the beta-carotene and nutrient levels of carrots from the different treatments.

Chapter 3

Results

Biomass

The experiment showed a statistically significant difference in above- and belowground biomass between the control group and those treated with both species of AMF. The belowground biomass of the carrots inoculated with both *R. clarus* and *R. intraradices* was 68.76% greater than the carrots grown without AMF ($F_{3,68} = 4.1877$; $P = 0.009$) (Figure 4). Carrots treated with single inoculum also showed an increase in belowground biomass, but these trends were not significant (*R .intraradices* vs. control $P = 0.133$; *R. clarus* vs. control $P = 0.072$). Aboveground biomass showed similar patterns with an increase of 112.38% between the plants inoculated with both AMF species and the control treatment ($F_{3,67} = 5.170$; $P = 0.003$) (Figure 5). Again, there was an increase in aboveground biomass between the carrots treated with each single AMF species and the controls but this trend was non-significant (Figure 5).

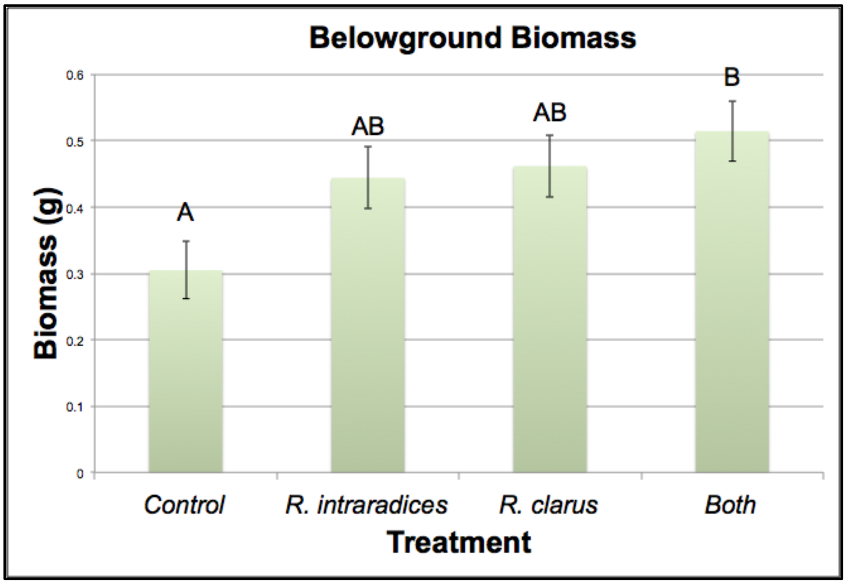

Figure 4: Carrots treated with both species of AMF increased belowground biomass by 68.76%.

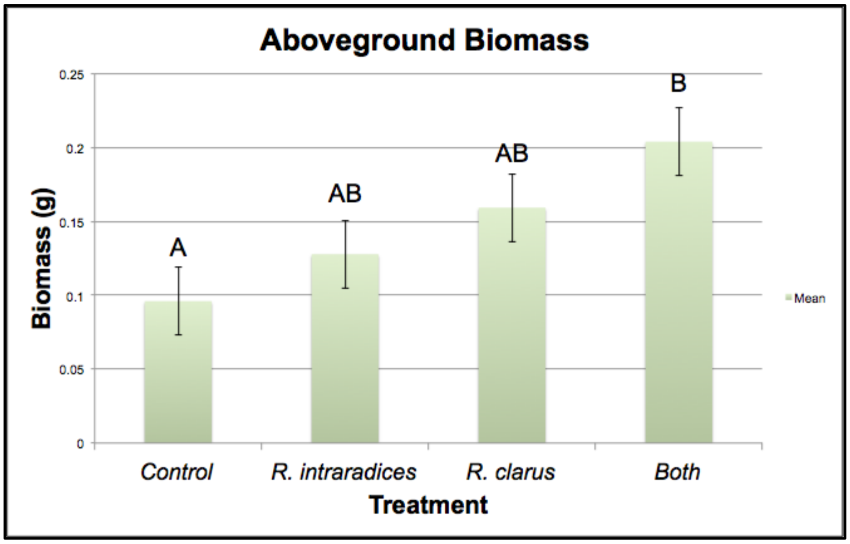

Figure 5: Carrots treated with both species of AMF increased aboveground biomass by 112.38%.

Nutrient Content

One-way ANOVA indicated that there was no significant difference in beta-carotene levels between controls and carrots inoculated with both AMF species ($F_{1,4} = 2.4643$, $P = 0.1915$). However, there was a trend that suggested an 18.28% increase in carrots treated with both species as compared to the control (Figure 6). A power analysis predicted that a modest increase in sample size to 12 would have given a significant result. Results from the nutrient analysis showed a significant increase in aluminum, phosphorus, and zinc levels with carrots inoculated with AMF (Table 1; Figures 7-8). Although the ANOVA was not statistically for difference in iron levels, there was also a notable trend for increased iron in carrots grown with AMF (Figure 9). There was a decrease in molybdenum and sodium (Table 1).

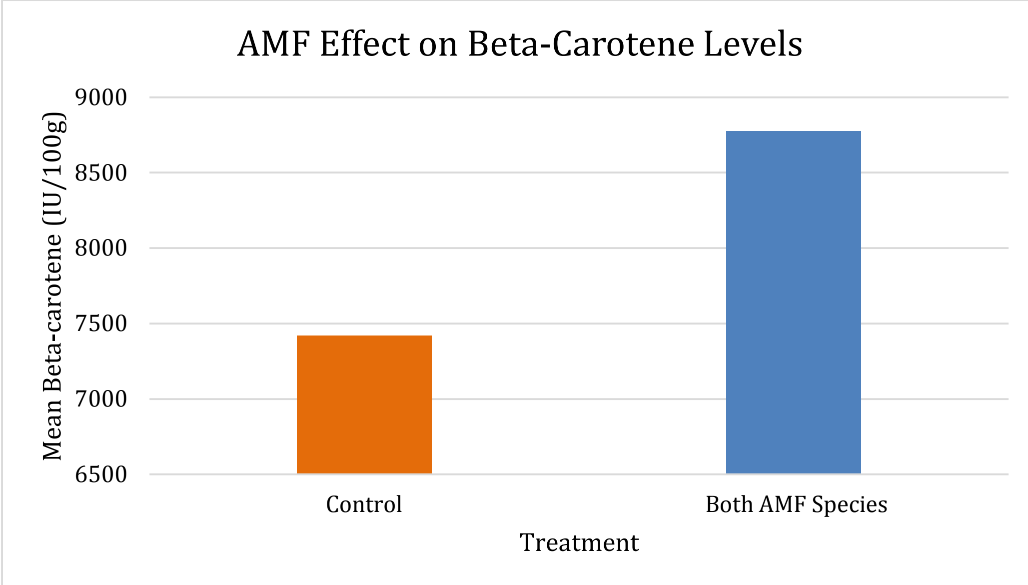

Figure 6. Levels of beta-carotene for the control and both AMF species inoculum treatment.

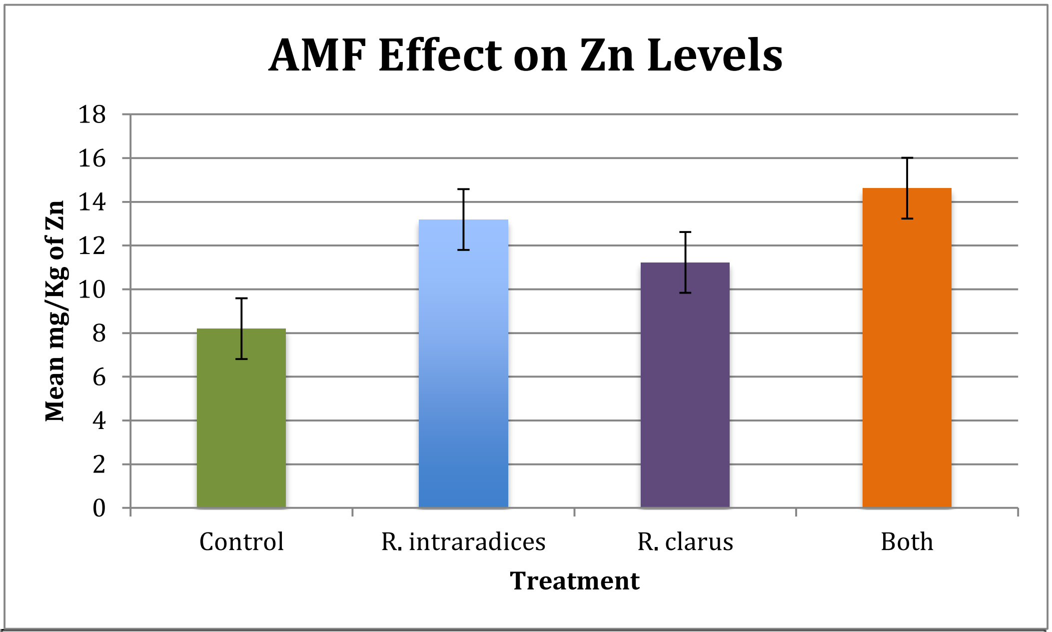

Figure 7: Comparison of mean levels of zinc between the four treatments.

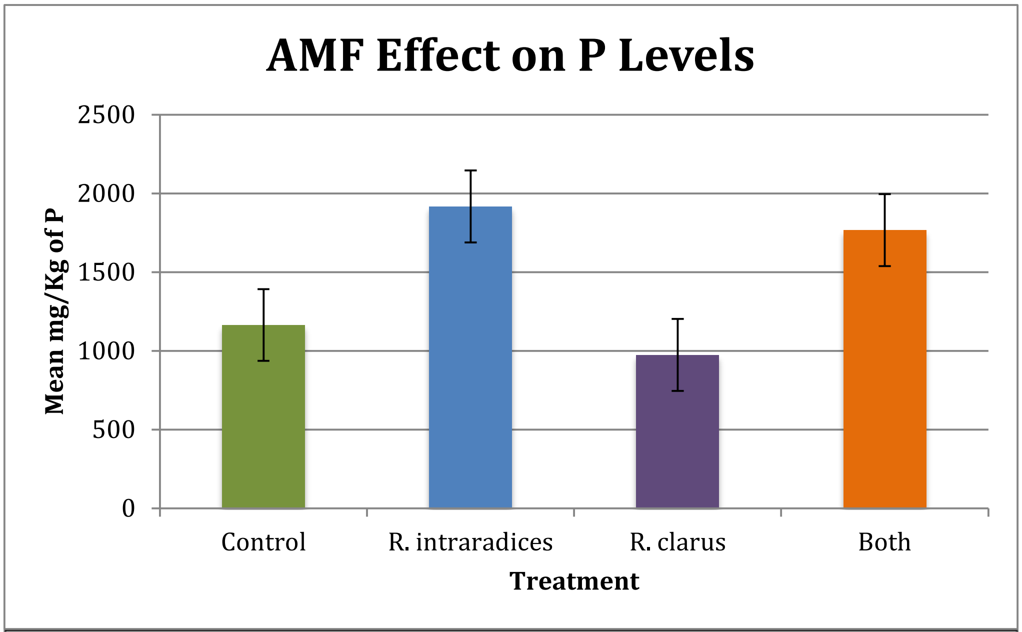

Figure 8: Comparison of mean levels of phosphorus between the AMF treatments.

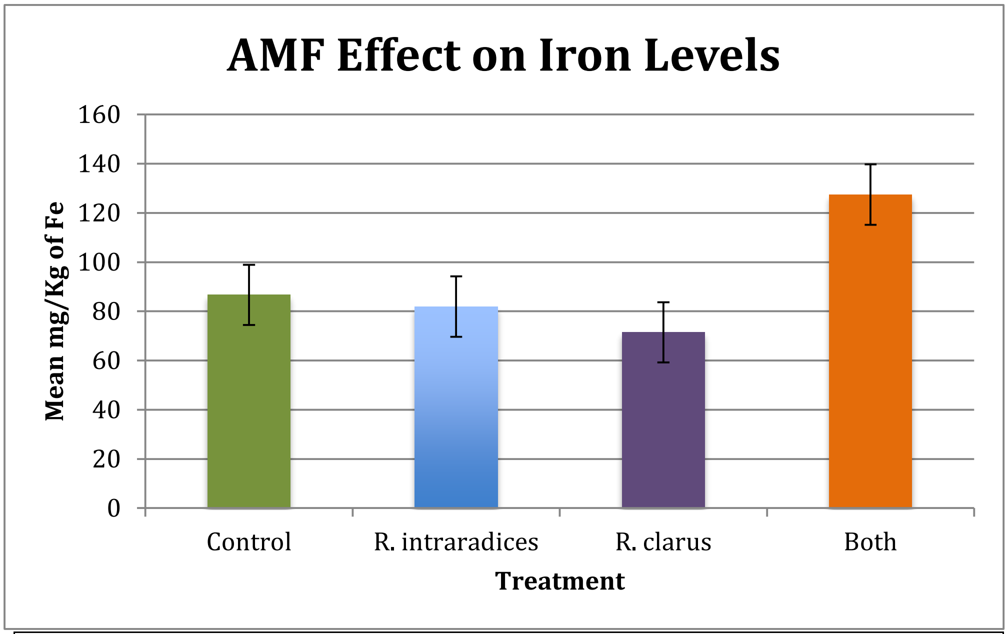

Figure 9: Comparison of the mean levels of iron between AMF treatments. These results were non-significant.

Table 1: One way ANOVA of nutrient content. Values in bold denote statistically different content levels between carrots from the control and inoculum with both AMF species.

Nutrient	F Ratio	DF	P-Value
Al	**4.0335**	**3, 19**	**0.0224**
B	1.093	3, 19	0.3762
Ca	1.0558	3, 19	0.391
Cu	0.8455	3, 19	0.4859
Fe	2.4199	3, 19	0.097
K	2.3698	3, 19	0.1027
Mg	1.5469	3, 19	0.235
Mn	0.439	3, 19	0.7277
Mo	**3.7618**	**3, 19**	**0.0283**
Na	**3.682**	**3, 19**	**0.0303**
P	**20.7547**	**3, 19**	**0.0001**
Zn	**6.6016**	**3, 19**	**0.003**

Chapter 4

Discussion

The mutualistic relationship between arbuscular mycorrhizal fungi and crop plants has the potential to increase the quality of crops. The mutualism has been understood for some time; however, little research has focused on how AMF affect crop nutritional content relevant to *human* health. Human health is dependent on the essential micro and macronutrients received from crop plants. Therefore, increasing the content of vitamins and minerals is of great interest to the security of the global food supply and overall health of society. Here I show that the inoculation of fungi increases the biomass and nutrients important to human health in carrots. By improving the quality of a crop, more nutrients can be obtained from a single serving. Therefore, high quality crops reduce some of the pressure to increase food production and will aid in sustaining global food supply.

The results suggest that the mutualism between plants and AMF has the potential to affect the nutritional quality of plants. In the present study, AMF inoculation shows trends of increased beta-carotene levels, an important precursor to vitamin A. Humans convert beta-carotene from plants to the active forms, retinol and retinoic acid, for use by the body. Vitamin A deficiencies are associated with immune infections, blindness, and lesions, and are the primary cause of childhood mortality in the developing world (Ejaz and Latif 2010). There was a trend of increasing beta-carotene when both *R. clarus* and *R. intraradices* were present. Although the results were not statistically significant, a power analysis predicted that a sample size of twelve would have produced significant results. A replicate study with more samples would most likely confirm AMF is a solution to increase the levels of this important pro-vitamin A carotenoid in carrots. If AMF increase can increase beta-carotene content per gram in carrots, there is huge potential to lower nutrient deficiencies globally.

In addition to beta-carotene, the results showed an increase in the average levels of aluminum, zinc, and phosphorous in carrots and also indicated trends in increased iron. As mentioned previously, these are important nutrients for human health and deficiencies can impair everyday life. Iron and zinc deficiencies are extremely prevalent around the globe. Iron is an important component to keeping cells oxygenated and a deficiency results in anemic conditions with symptoms including fatigue, delayed growth, and inability to focus. Zinc is an important mineral for a healthy immune system and the mobility of zinc in soils is very low and its uptake by organisms is diffusion-limited (Vallee and Falchuk 1993; Frossard et al. 2000; Smith and Reed 2008). The ability of AMF to sequester these micronutrients in the soil and increase the content in the edible portion of the crop is exciting and should be a focus of future research. AMF inoculation had the opposite effect on molybdenum and sodium. The levels were decreased when compared to control carrot levels. Possibly, the AMF are not able to aid in transferring the nutrients to the carrot because the fungi use those specific resources for themselves (Smith and Reed 2008). Regardless, AMF use in agriculture can have a big impact on crop nutrient content and research should continue to look for ways to produce the highest nutrient crop.

In addition to an increase in nutrient content, the present study also finds that AMF increases both the above- and belowground biomass of carrots. I observed a 68.76% increase of belowground biomass and a 112.38% increase in aboveground biomass in carrots grown with both species of AMF. This finding is corroborated by past studies that show AMF increase carrot production. Affokopon et al. (2010) showed that application of AMF in field experiments increased yield more than 300% as compared to non-AMF controls. The percent increase should be of particular economical interest for farmers; the more biomass produced from a single seed the better. Increased aboveground biomass can concomitantly increase photosynthetic activity by the plant. In turn, increases in photosynthetic activity means that plants can offer more carbon to the fungi and receive more rewards in return (Kiers et al. 2011). Future research should be focused on testing what combination and amount of AMF species can increase the photosynthetic activity of the plant and provide the fungi with more food to manipulate a reciprocal benefit from the fungi to the plant.

The findings from this study showed that there was a significantly larger benefit observed when both *R. clarus* and *R. intraradices* were applied, suggesting enhanced nutrient status with a diverse community of fungi verses single isolates. The results from the ANOVA correspond to previous studies that acknowledge that specific AMF species combinations produce differing benefits for the plant (van der Heijden and Scheublin 2007). These benefits are possibly enhanced via specialization of the AMF. For instance, some AMF species may specialize on obtaining a specific nutrient for their host plant (Hart and Reader 2002). Alternatively, specific AMF-plant species combinations can be more efficient for nutrient acquisition (Hausmann and Hawkes, 2010; Wardle et al. 2004). In the present study, it seems that specialization in nutrient acquisition might explain the enhanced performance of carrots with both AMF species. This is supported by a recent study that identified the diversity of AMF and their roles in ecosystems (Lee et al. 2013). They found different community compositions of AMF affect plants differently because different species of AMF have different effects on various aspects of the symbiosis. Therefore, to maximize the benefits to agriculture productivity, it is essential to determine complementary combinations of inoculum for specific crop species.

An important aspect of the results is that the benefits derived from AMF were observed in exceedingly poor soil conditions. The fungi were successful at increasing nutrients in sterile sand, suggesting that AMF could be a realistic solution for improving crop production in extremely nutrient deficient soils. Deficient soils are common in underdeveloped countries that struggle with hunger issues. There is a strong correlation between nutrient deficient soils and hidden hunger. Hidden hunger occurs when people do not meet their nutrient requirements because the food is deficient in micronutrients. Therefore, the ability to grow nutrient dense crops in these poor soil conditions opens up the opportunity to decrease micronutrient and macronutrient disorders and aid in relieving world hunger.

Chapter 5

Conclusion

Together, the results of my study and previous research suggest that AMF inoculation is a viable solution to improve sustainable agricultural practices and improved food quality. There is an urgent need for agriculture to produce enough food of high nutritional quality and diversity to satisfy a balanced diet for all people. Traditional methods of increasing yields have been focused on increasing calorie bulk of staple crops, such as wheat and rice, and the use of harmful pesticides and fertilizers. The use of harmful chemicals to increase yields in nutrient poor soils has detrimental effects. The fertilizer industry is responsible for about 1.2% of the world global greenhouse gas emissions with 90% from the production of ammonia (NH_3) (Swaminathan and Sukalac 2004). Nitrous oxide (N_2O), which according to the Environmental Protection Agency has a greater impact compared to CO_2 on the warming of the atmosphere, is emitted when nitrogen is added to the soil through synthetic fertilizers. The application of synthetic fertilizers results in nitrogen run off in waterways causing contamination of drinking water, which, if ingested, reduces our ability to carry oxygen. The release of oxidized nitrated from the application of fertilizer also contributes to the formation of smog, greenhouse gasses, and the destruction of the ozone layer. AMF can act as an environmentally safer application to produce more food with higher nutrient quality in poor soil conditions.

On top of the use of harmful chemical, the current agricultural system has placed itself in a vulnerable position by reducing the diversity of crops. The agricultural methods prioritizing monoculture systems aim to increase cereal yields creates major micronutrient deficiencies by creating an off-balance ratio of macronutrients and a diminished level of micronutrient (Welch 1995). A balanced diet is fundamental for ensuring healthy and productive lives. If you look at

Maslow’s hierarchy of needs, food is at the base of the pyramid. Therefore, without proper nutrition, humans cannot reach the higher levels such as security, social relationships, self-esteem, and important problem solving/creativity skills. Without the energy to obtain the higher level needs, human’s benefit to society decreases. Malnutrition affects about 40% of the world’s people, many from developing nations (Schuftan et al. 1998). In order for developing nations to establish globally, it is vital to receive proper nutrition to ensure full cognitive and physical capacity. A well balanced diet includes proper macronutrient and micronutrients. Micronutrients include essential elements and vitamins that the body cannot produce and are required for humans. There are 49 nutrients required to meet human metabolic needs and inadequate consumption of even one of these nutrients will result in metabolic disturbances that can lead to poor health, impaired development, and large economic costs to society (Branca and Ferrari, 2002; Ramakrishnan *et al*., 1999; Gordon 1997). Human rely on the agricultural system to provide enough products containing adequate quantities of all nutrients during all seasons.

References

Affokpon, A., Coyne, D. L., Lawouin, L., Tossou, C., Agbede, R. D., & Coosemans, J. (2011). Effectiveness of native West African arbuscular mycorrhizal fungi in protecting vegetable crops against root-knot nematodes. *Biology and Fertility of Soils, 47*(2), 207-217. doi:10.1007/s00374-010-0525-1

Bender, S. F., & van der Heijden, M. G. A. (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology, 52*(1), 228-239. doi:10.1111/1365-2664.12351

Branca, F., & Ferrari, M. (2002). Impact of micronutrient deficiencies on growth: The stunting syndrome. *Annals of Nutrition and Metabolism, 46*, 8-17. doi:10.1159/000066397

Brundrett MC, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with mycorrhizae in forestry and agriculture. Canberra, Australia: ACIAR Monograph 32.

Cavagnaro, T. (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. *Plant Soil*, 304, 315-325.

Clark, R.B., & Zeto, S.K. (2000). Mineral acquisition by arbuscular mycorrhizal plants. J. *Plant Nutr.*, 23, 867-902.

N. Dechassa, M.K. Schenk, N. Claassen, B. Steingrobe (2003) Phosphorus efficiency of cabbage (*Brassica oleracea* L. var. *capitata*) carrot (*Daucus carota* L.), and potato (*Solanum tuberosum* L.) *Plant Soil*, 250 (2003), pp. 215–224

Ejaz MS, Latif N. Stunting and micronutrient deficiencies in malnourished children. J Pak Med Assoc. 2010;60:543–47.

Frossard E, Condron L M, Oberson A, Sinaj S and Fardeau J C 2000 Processes governing phosphorus availability in temperate soils. J. Environ. Qual. 29, 12–53.

Gardner, G. T., Halweil, B., & Peterson, J. A. (2000). *Underfed and overfed: The global epidemic of malnutrition*. Washington, DC: Worldwatch Institute.

Gordon, N. 1997 Nutrition and cognitive function. *Brain Dev.* 19: 165-170.

Hart, M. M., & Forsythe, J. A. (2012). Using arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. *Scientia Horticulturae*, *148*(0), 206-214

Hart, M., & Reader, R. (2002). Does percent root length colonization and soil hyphal length reflect the extent of colonization for all AMF? *Mycorrhiza, 12*(6), 297-301. doi:10.1007/s00572-002-0186-5

Hausmann NT, Hawkes CV. 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. *New Phytologist* 183: 1188–1200.

Henao, J. and Baanante, C. 2006. *Agricultural Production and Soil Nutrient Mining in Africa Implications for Resource Conservation and Policy Development*. IFDC, Muscle Shoals, Alabama.

Johansen, A., Finlay, R.D., & Olsson, P.A. (1996). Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus Glomus intraradices. *New Phytol*., 133, 705-712.

Karagiannidis, N., Nikolaou, N., Ipsilantis, I., & Zioziou, E. (2007). Effects of different N fertilizers on the activity of *Glomus mosseae* and on grapevine nutrition and berry composition. Mycorrhiza, 18, 43-50.

Karban, R., Brody, A., & Schunatnorst, W. (1989). Crowding and a plant ability to defend itself against herbivores and diseases. *American Naturalist, 134*(5), 749-760. doi:10.1086/285009

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., . . . Buecking, H. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science, 333*(6044), 880-882. doi:10.1126/science.1208473

Kim, K., Yim, W., Trivedi, P., (...), & Sa, T. (2010). Synergistic effects of inoculating arbuscular mycorrhizal fungi and Methylobacterium oryzae strains on growth and nutrient uptake of red pepper (Capsicum annuum L*.). Plant Soil*, 327, 429-440.

Lambers, H., Mougel, C., Jaillard, B., & Hinsinger, P. (2009). Plant-microbe-soil interactions in the rhizosphere: An evolutionary perspective. *Plant and Soil, 321*(1-2), 83-115. doi:10.1007/s11104-009-0042-x

Lee, E., Eo, J., Ka, K., & Eom, A. (2013). Diversity of arbuscular mycorrhizal fungi and their roles in ecosystems. *Mycobiology, 41*(3), 121-125. doi:10.5941/MYCO.2013.41.3.121

Leigh, J., Hodge, A., & Fitter, A.H. (2009). Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytol.*, 181, 199-207.

Loladze, I. (2002). Rising atmospheric CO2 and human nutrition: Toward globally imbalanced plant stoichiometry? *Trends in Ecology & Evolution, 17*(10), 457-461. doi:10.1016/S0169-5347(02)02587-9

Medina, A., Roldan, A., & Azcon, R. (2010). The effectiveness of arbuscular-mycorrhizal fungi and *Aspergillus niger* or *Phanerochaete chrysosporium* treated organic amendments from olive residues upon plant growth in a semi-arid degraded soil. *Journal of Environmental Management, 91*(12), 2547-2553. doi:10.1016/j.jenvman.2010.07.008

O'Donoghue, Erik J., Robert A. Hoppe, David E. Banker, Robert Ebel, Keith Fuglie, Penni Korb, Michael Livingston, Cynthia Nickerson, and Carmen Sandretto. *The Changing Organization of U.S. Farming.* EIB-88. U.S. Dept. of Agriculture, Econ. Res. Serv. December 2011.

Paradi, I., Bratek, Z., & Lang, F. (2003). Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*. *Biologia Plantarum*, *46*(4), 563-569.

Plenchette, C., Clermont-Dauphin, C., Meynard, J. M., & Fortin, J. A. (2005). Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science, 85*(1), 31-40.

Ramakrishnan, D., J Salim, and W.R. Curtis. 1999. Monitoring biomass in root culture systems. *Biotechnology and Bioengineering* 62(6): 711-721.

Schuftan, C., Ramalingaswami, V., & Levinson, F. (1998). Micronutrient deficiencies and protein-energy malnutrition. *Lancet, 351*(9118), 1812-1812. doi:10.1016/S0140-6736(05)78774-2

Smith, S. E. (2008). *Mycorrhizal symbiosis*. New York: Academic Press.

Swaminathan, B., & Sukalac, K. E. (2004). Technology transfer and mitigation of climate change: The fertilizer industry perspective. Presented at the IPCC Expert Meeting on Industrial Technology Development, Transfer and Diffusion, Tokyo, Japan, 21–23 Sept. 2004.

Underwood, B.A. 1998 From research to global realtity: the micronutrient story. J Nutr 128:145-151.

Vallee B., Falchuck K. (1993). The biochemical basis of zinc physiology. *Physiological Reviews*, *73*(1), 79-118.

van der Heijden, M. G. A., & Scheublin, T. R. (2007). Functional traits in mycorrhizal ecology: Their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist*, *174*(2), 244-250. doi:10.1111/j.1469-8137.2007.02041.x

Veresoglou, S.D., Shaw, L.J., & Sen, R. (2010). *Glomus intraradices* and *Gigaspora margarita* arbuscular mycorrhizal associations differentially affect nitrogen and potassium nutrition of Plantago lanceolata in a low fertility dune soil. *Plant Soil*, 340, 481-490.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setala, H., van der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, *304*(5677), 1629-1633. doi:10.1126/science.1094875

Welch, R. M., & Graham, R. D. (1999). A new paradigm for world agriculture: Meeting human needs: Productive, sustainable, nutritious. *Field Crops Research, 60*(1–2), 1-10.

Appendices

"Eating is Cultural Act: Notes on Rhizomes, Deserts and Ugly Fruit," Presented by the Canary Lab 12/7/15

“AMF: The Future to Sustainable Agriculture
Margo Malone, Lily Fein, Ryan Pierson

Ecology deals with relationships. This relationship begins with a tiny spore. The relationship is mutually beneficial. The spore of the Arbuscular mycorrhizal fungi, AMF for short, germinates and produces hyphae that enter between the plant cell walls. The fungi extend through the soil and provide nutrients otherwise untapped in return for carbon delivered from the plant. The AMF act like fiber optic cables, carrying information and metabolites between plants and warning neighbors of herbivore attacks, threatening pathogens, and impending droughts.

The AMF have the potential to sustain our future agricultural system. They allow the plant to grow in harsh conditions. Implanted in urban settings, AMF vesicles store soil toxins away from the edible crop product. Communication between the crops allows the farmer to use less pesticides and fertilizers. AMF means more plant nutrients, a better crop yield, and a more efficient food system. “

“Take-home AMF”

Final exhibition product.