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# The Effect of Rhizophagus intraradices Mycorrhizal Fungus on Iron Uptake of Corn: A Global Overview on the Effects of Climate Change on the Nutritional Content of Crops

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## INTRODUCTION

Arbuscular mycorrhizal fungi form symbiotic relationships with vascular plants. They inhabit the root cells (vesicles) of the plant as well as create hyphae threads into the soil. Through these threads, the fungi can transform macronutrients into forms usable by the vascular plants and trade these nutrients for sugars in return. Figure 1 shows a stained sample of a root inhabited by a type of arbuscular fungi (Pei, Y. et al. 2020).

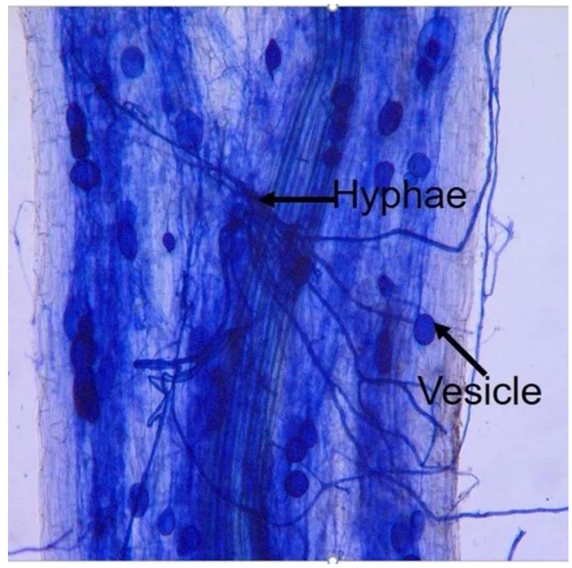


***Figure 1: A stained root sample showing the vesicles and hyphae of an arbuscular fungus (Pei, Y. et al. 2020).***

Certain types of mycorrhizal fungi can increase the nutrient uptake of plants, so mycorrhizal fungi could influence the macronutrient availability of the produce we eat. For example, a previous study looked specifically at the ability of the arbuscular fungus *Rhizophagus irregularis* to act as a transporter for copper, iron, and zinc macronutrients by analyzing its genome (Lehmann & Rillig 2015). It was found that *R. irregularis* contained genetic sequences specific to changing unusable ferric hydroxide naturally occurring in soils into a usable form of iron for plants (Lehmann, A., & Rillig, M. C. 2015).

In this study, we used a related arbuscular mycorrhizal species known as *R. intraradices,* chosen for its known ability to improve plant nutrition (Lehmann, A., & Rillig, M. C. 2015; Tamayo, E et al. 2014), and corn, which represents a large portion of the globally produced and required crops (CropLife International, 2021). While there is significant research showing positive relationships between mycorrhizal fungi and plants (Bona, E. et al. 2016; Saia, S. et al. 2020; etc.), there is little research connecting the potential for these fungi to improve the overall nutritional content of resulting produce with the intention of also improving global health (but see Barazetti, A.R. et al. 2019; Lehmann, A. & Rillig, M.C. 2015, etc.).

## METHODOLOGY

### SEEDS

Maize seeds of the Golden Bantam 8 Row variety, which was expected take 100 days to harvest, were obtained and planted at a density of two seeds per 5-gallon bucket. This variety can be planted at 2 stalks per square foot (or 2 stalks per 5-gallon bucket), each stalk producing approximately 2 to 3 large ears. Samples were categorized in two groups: those with mycorrhizal fungi introduced (treatment), and those without (control). To obtain a good sample size, we grew 50 stalks of each treatment, yielding 100 stalks overall, with up to 200-300 ears of corn expected at harvest time for assessment.

### PREPARATION AND STERILIZATION

A total of 50 buckets (5 gallons each) underwent a cleaning and sterilization process. They were first scrubbed to remove all buildup, then rinsed. Then they were washed with a cream cleanser product (Bon Ami), and again rinsed. Lastly, they were sterilized with a 2% bleach solution (1 part 6% bleach solution and 2 parts water) and rinsed again.

The soil (a greenhouse blend: peat moss, perlite, sand, mulch, vermiculite, fine bark, coconut fiber, diatomaceous earth, pumic, aged fir bark) was also sterilized. We used one plastic scoop for regular soil media, and one for sanitized soil media. The substrate was scooped into Pyrex brand glass bakeware and heated in a drying oven at 200° Fahrenheit for 30 minutes and then transferred to sterilized buckets with a sanitized scoop. These were covered with parchment paper and moved to greenhouse space to await the next steps. Seeds were sterilized using a 70% ethanol solution. The seeds were soaked in the solution for 5 minutes before being planted at a density of two seeds per bucket. The inoculated corn samples received 10 ml per bucket of a culture of mycorrhizal fungi in a sandy substrate (obtained from West Virginia University) added approximately ¼ inch below the surface of the soil.

Plants were kept under natural lighting and cooled with an evaporative cooling system. Watering occurred a minimum of 3 times per week, with more watering occurring during high heat days/weeks. Fertilizer started in the final weeks and consisted of a low phosphorus fish emulsion fertilizer mixed into the water supply.

Approximately 3 weeks into growth, several stalks were failing to hold themselves up. They were moved to an area of the greenhouse with more sunlight, and every stalk was stabilized with a bamboo stake.

We expected to test for the establishment of mycorrhizal fungi by visually assessing for mycorrhizal hyphae as well as dying and ubserving roots under the microscope, as arbuscular fungi penetrate the cells inside the roots of the plants with which they are in symbiotic association. At the end of summer/beginning of fall, crops were to be harvested and seeds would be tested for iron content.

## RESULTS

Nearing the last few weeks of growth, I noticed an infestation of corn leaf aphids had started. They were laying eggs on nearly every stalk of control corn. However, not a single inoculated stalk had live aphids or eggs. This continued to be true for nearly two weeks, despite the pots sitting very near each other. Further research was completed, which is more fully described in the discussion section of this paper.

The corn plants grew relatively well earlier in the summer; stalks were growing at approximately two feet per month and developed pollination tassels in a timely manner. Ears also started to develop at an appropriate time. However, as the harvest date neared, it became apparent that the corn plants were no longer thriving. They seemed to have experienced stunted growth, were discolored, and had produced smaller ears than expected. Further inspection showed that while there were ears to harvest, each did not contain enough kernels for data analysis to show results. The inoculated roots did not show evidence of hyphae that would indicate successful symbiosis. Therefore, it was decided that sample analysis for iron content would not be useful. In the next section, we highlight some of the potential setbacks that hindered this project.

## SETBACKS

### IRREGULAR AND INSUFFICIENT IRRIGATION

While greenhouse visits were frequent (approximately 3-5 days per week), it was clear that the pots had excessive drainage. By the end of the summer and into the early fall, the soil was very dry at the start of every visit.

### NO DIRECT SUNLIGHT

Various studies that have used corn as a sample crop showed greater success when growing outside with frequent, direct sunlight hours. The greenhouse provided ample natural light; however, the light was dispersed. Also, because of the physical structure of the greenhouse, shade could vary.

### NO WIND

Some studies show that certain plant types, including corn, benefit from the motion created by wind moving through their leaves and lightly shaking their stems/stalks. This was not possible within the greenhouse setting.

ADDITIONAL FERTILIZATION

As per fertilizer directions and the advice of other greenhouse researchers, the corn was fertilized towards the beginning of what would have been the harvest cycle. However, it seemed as though the plants could have benefited from earlier or additional fertilization, perhaps even a month before it was applied. This may be due to the greenhouse setting or potentially the fact that the soil had been baked and lost some nutrients.

## DISCUSSION

This research could bridge a gap between these global health and improved agricultural practices with the potential to have significant contributions to the agricultural communities as well as groups facing food insecurity and lack of availability of nutritionally rich foods. Corn/maize has been deemed a global "staple crop," which means that it makes up a major or dominant part of a population's diet, is a primary source of nutrition, and is eaten on a regular basis (National Geographic Education Staff, 2014). Additionally, corn has long been a cultural keystone food for many cultures, particularly in central and south American countries, where indigenous wild varieties can be found. These also happen to be countries that are growing a large portion of the world's corn and continue to suffer the effects of chemical fertilizers and climate change (Entwistle, B. 2005).

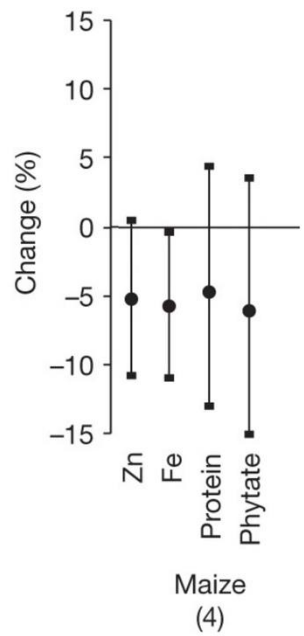


*Figure 2: A figure from the research of Dr. Samuel Myers (Harvard) showing the percent decrease of several macronutrients in maize crops when subjected to heightened CO conditions, levels predicted to be reached at the middle of this century (Myers, S. S., et al. 2017).*

Certain fungal species have genome sequences that help transform iron to be able to be taken up by plants. Anemia represents the most prevalent worldwide nutritional disorder, with over 50% cases due to iron deficiency (Stoltzfus, R. J. 2003). Together, this means that certain fungal strains may be able to alleviate some of these health effects, particularly in the face of climate change. It is estimated that at least two billion people globally are suffering deficiencies of one or more major macronutrients. Undernutrition accounts for approximately three million deaths of children annually, nearly half of global child deaths (Stoltzfus, R. J. 2003). 59% of the global totals of children ages 1-5 and women of childbearing age live in countries with anemia rates exceeding 20% of the total population. The intake of iron in these regions is already expected to diminish by at least 3.8% in direct relation to crop nutrient deficiencies due to emissions. FACE (Free Air Carbon Enrichment) experiments simulating elevated $CO_2$ effects on nutrient content of major crops showed that all major crops would face nutrient depletion, maize being no exception (Figure 2; Myers, S. et al. 2017).

Not only are these nutritional deficiencies already affecting vulnerable populations, but food access is known to be determined greatly by political and economic forces. This furthers the discrimination towards certain groups based on gender, caste and wealth, legal recognition of land and asset ownership, etc. All these factors may lead to more mass migrations and displacements, implications that have already greatly affected regions like sub-Saharan Africa and the Middle East (Myers, S. et al. 2017). While subsequent research will be extensive, this proposed project will start the conversation regarding the ability to use mycorrhizal fungi to address global malnutrition.

## PROPOSED IMPROVEMENTS

While this iteration of the research was unsuccessful, it clearly outlined parameters that should be revised for future attempts.  The implications are still relevant and forthcoming, so it is highly recommended that a revised version of this research be conducted as soon as possible.  To do so, we suggest following the proposed improvements suggested in the next section.

If this project is to be repeated in a greenhouse setting, there would need to be several improvements to the overall design.  The greenhouse placement was chosen to limit confounding factors of atmospheric chemicals including carbon.  It was also a way of avoiding the introduction of other strains of mycorrhizal fungi in the soil.  While field experiments will need to be conducted sometime during the development of these inoculants, the beginning stages should be as controlled as possible.  Therefore, to complete further research within a greenhouse, it is advised that researchers implement an irrigation system that allows for regular, daily dosing of water.  This could potentially occur multiple times in a day in regular amounts.  Grow lights may be used to mimic more direct sunlight within the greenhouse.  It may also be beneficial to simulate a breeze with fans or regular, manual shaking of the stalks.  More funding would be beneficial to employing these suggested improvements.

## PEST INFORMATION

Mycorrhizal fungi can send signals through their hyphae, thread-like strands that fungi send into the soil from a vascular plant's roots.  Using these hyphae, fungi can uptake macronutrients and quite frequently change the chemical environment, making unavailable nutrient forms more biologically available for uptake. In return, the plant sends the fungus energy in the form of carbohydrates. It is already well known that plants can send molecules into the atmosphere to communicate to one another. This research showed that when control plants were manually infested with aphids, they released a chemical called methyl salicylate. This chemical repels aphids as well as attracts parasitoid wasps, a natural predator to said aphids. Additionally, through the strands of the fungus introduced to some of the plant subjects, a signal was sent to prompt this production even further. Also highlighted was a phenomenon called “mycorrhizae-induced resistance”. This is the high resistance to both biotic and abiotic factors in plants that are mutualistic with mycorrhizal strains (Johnson, D., & Gilbert, L. 2014).

While there is little evidence that fungi can promote atmospheric signaling, it enhances the plant’s ability to do so by furthering chemical production in the rhizosphere. Researchers completed a study in 2001 to identify a link between the mycorrhizal fungi and the aerial transmission of methyl salicylate. However, it was concluded that further research was needed as they were only able to confirm the previous hypothesis that the plants create the chemical and send it out into the atmosphere to each other, and the fungi merely enhance underground signaling (Chamberlain, K., et al. 2001).

All of this signaling ability is irrelevant if it doesn’t move quickly. Aphids reproduce a lot, and they reproduce fast. Therefore, these plants need to send warning signals as quickly as possible. A final study published in 2013 showed that these signals can start as soon as within the first 24 hours following infestation. The repellent becomes active within 24 hours, and only becomes stronger with more time (Babikova, Z., et al. 2013, 2).

It is likely that the crops assessed in our research were conducting this type of signaling with methyl salicylate. As the infection spread throughout the control crops, the inoculated crops were able to resist the aphids for much longer, nearly 2 weeks. This potentially shows evidence of the phenomenon and yet another reason why inoculating crops with these types of fungi strains can and will be beneficial to global crop production. Further research can be done to determine if the inoculants can be optimized to aid these crops in pest resistance.

## FUTURE RESEARCH

Once an improved version of this project is completed and data is analyzed, it is expected that we would find improved availability of macronutrients in produce for consumption. We would then be able to move into field experiment phases to observe the potential success of these inoculants in a relevant setting to where they would be used once available for implementation on larger agricultural scales. It would be necessary to observe their potential for symbiosis while other strains are available, as well as their ability to improve nutrient availability and uptake in naturally occurring soils. Analysis of the potential life years gained from the approximate percent macronutrient improvements could determine whether this solution is potent enough to counteract the effects of climate change we are expecting to see in the next few decades.

## IMPLICATIONS

The health effects of climate change are broad and quickly growing. A recent assessment by NASA showed that the effects of climate change on corn and wheat crops could become dire as soon as 2030 (Gray, E. 2021). The study we are conducting here has the potential to improve global agricultural systems in a way that directly relates to the potential for improved health.

With these outcomes, we may begin to assess how to optimize mycorrhizal inoculants to be used as means of improving crop nutrition and yield. Variations in fungi strains may show adaptability to climate, nutrient type, crop type, etc. These implications will affect policies overseeing agricultural sites, restoration sites, and further both environmental research initiatives and the relationships between researchers and crop producers. This research also holds significant weight in the global agricultural economy and can directly improve malnutrition rates which are particularly dire in non-industrialized nations. Once implemented, these inoculants may relieve pressures on global healthcare systems as well. It is essential we begin to look at holistic means of improvement human health in the face of climate change, and in doing so we will start to mend our relationship with and the planet itself.

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