Abstract

Soybean (*Glycine max L.*) is one of the world's most economically important crops. In America alone, soybeans dominate a full third of agricultural production and serve as a principal export. In what ways can we effectively maintain, manage, and magnify the soybean? Classical methods employ the bare necessities, sunlight, water, nutrients, favorable temperatures, humidity, etc, maybe even fertilizers and crop rotations, but an unlikely soybean ally may prove even more effective. More than 95% of terrestrial plants form symbiotic, mutualistic relationships with specialized fungi known as mycorrhiza. Through the inoculation of these mycorrhizal fungi, factors such as the morphology, growth rate, physiological tolerance, etc. improve drastically. My study aims to answer the question: "Would Arbuscular Mycorrhizal Fungi Inoculation Positively Impact the Growth and Physiological Tolerance of Soybean Plants In Drought AND Flood Conditions?" My results state it just might. Significant p-values for shoot dry mass in desiccated groups ($p = 0.007072$), regular ($p = 0.011882$), and abundant groups ($p = 0.022543$) indicated that inoculation likely played a role in the discrepancies between groups. That said, the regularly water-treated groups ($p = 0.024437$) significantly increased in root dry mass when inoculated. However, the non-significant p-values of root dry mass in desiccated ($p = 0.327979$) and abundant groups ($p = 0.1142$) were not significant. The significant p-values (Desiccated $p = 0.031859$, Regular $p = 0.02373$, Abundant $p = 0.013704$) obtained from the total chlorophyll values indicate that inoculation does improve physiological water tolerance.

Introduction

Growing healthy plants is important for a variety of reasons. Not only do healthy plants look aesthetically pleasing, but they are also better able to withstand pests and diseases, and can provide us with food, oxygen, and medicine. There are several methods that can be used to help promote the growth of healthy plants. These include providing plants with proper sunlight, water, and nutrients, as well as maintaining a suitable temperature and humidity. Another common method is the use of fertilizers, which are substances that contain a range of nutrients that plants need in order to thrive. When applied to the soil, fertilizers can help to improve the availability of these nutrients to the plant, which can lead to healthier growth and development. One particularly effective method for promoting healthy plant growth is the use of mycorrhizal fungi. These fungi form a symbiotic relationship with the roots of plants, helping to improve the uptake of water and nutrients, and increasing the plant's resistance to stress.

Mycorrhizal fungi can be a more effective tool for growing healthy plants than fertilizers for several reasons. First, mycorrhizal fungi form a symbiotic relationship with the roots of plants, providing them with increased access to water and nutrients. This can be especially beneficial in soil that is poor in nutrients or has a low water-holding capacity, as the fungi are able to help the plants obtain the resources they need to thrive. In contrast, fertilizers only provide plants with a limited range of nutrients and do not improve the availability of water. Second, mycorrhizal fungi are able to improve the structure and fertility of soil over time, as they help to break down organic matter and release nutrients back into the soil. This can lead to more sustainable and healthy plant growth in the long term. Fertilizers, on the other hand, can potentially lead to soil degradation if used excessively, as they can alter the chemical balance of the soil and potentially lead to the leaching of nutrients. Finally, mycorrhizal fungi are generally considered to be more environmentally friendly than synthetic fertilizers, as they are natural and do not rely on the use of chemicals. This can make them a better choice for those who are looking for more sustainable gardening practices. Overall, while fertilizers can be a useful tool for promoting healthy plant growth in some situations, the use of mycorrhizal fungi can provide a more holistic and sustainable solution for many growers.

More than 95% of terrestrial plants form symbiotic, mutualistic relationships with specialized fungi known as mycorrhiza. (Rigamonte et al., 2010). There are two main types of mycorrhizal fungi: endomycorrhizae and ectomycorrhizae. Endomycorrhizae penetrate the cell walls of the host plants using thread-like fibers called hyphae. Ectomycorrhizae, on the other hand, do not penetrate the cells and instead spread their hyphae across the surface of the walls. Arbuscular mycorrhizal fungi (AMF) are endomycorrhizae as they penetrate root cells and form systems of arbuscules. By infecting the host plants' roots, mycorrhizal fungi facilitate the host plants' roots' absorption of necessary water and mineral nutrients, while also acting as a barrier to improve resistance and tolerance to biotic and abiotic stress factors. It's due to these factors that host plants inoculated with AMF have significantly higher rates of growth, especially in ultramafic, nutrient-poor, or moisture-poor soils (Bourles et al., 2020). Mycorrhizal fungi powders and liquids are available commercially and industrially for projects ranging from at-home plant sanctuaries to large-scale conventional and organic farms. This study compares the growth and physiological water tolerance of mycorrhizae-infected and mycorrhizae-uninoculated soybean plants in order to establish the benefits of mycorrhizal fungi-root relationships. It was hypothesized that mycorrhizae-inoculated soybean plants will be more resistant to pathogen infection and experience higher rates of growth than uninoculated plants.

Mycorrhiza helper bacteria (MHB) are bacterial strains that positively influence the establishment and functionality of mycorrhizal symbiosis. They (i) promote AMF spore germination, (ii) stimulate mycelial growth, (iii) change soil properties, (iv) produce stimulatory signals and allow the recognition of fungi by plants, and finally (v) enhance the colonization of roots by fungi. Alexandre Bourles' study showed that co-inoculation with both AMF and MHB was necessary to improve the growth of *Tetraria comosa* in ultramafic Caledonian soil that are characterized by low levels of major plant nutrients (N, P, K), strong unbalanced Ca/ Mg ratios, and the presence of iron oxides with high levels of metals (Ni, Co, Cr, Mn) (2020). Otherwise, the plant's growth would be negatively affected by the addition of the AMF alone. Although, other studies, such as Valentina Fiorilli's on wheat yield and resistance to leaf pathogen, *Xanthomonas translucens,* when inoculated with AMF alone, show that results with solo-inoculation are inconsistent (2018). That said, the inhibiting effects of the fungi on pathogens may have led to a comparatively higher growth rate. Since there are many differences in methods, plant species, AMF species, MHB species, and growth metrics, more research may be required to identify the cause(s) of these inconsistencies.

Endomycorrhizal AM symbiosis form morphotypes of either Arum- or Paris-type depending on the host plant species. Arum-type colonization utilizes branching filaments called hyphae to penetrate inner cortical cells of root systems, resulting in highly branched arbuscules. Paris-type colonization spread hyphae via intracellular passages of cortical cells, forming coil-like arbuscules (Tominaga et al., 2022). Arbuscules are the site of nutrient transfer between the plant and the fungus and are therefore of crucial importance for AM symbiosis. It is here that the AMF provides the host plant with the surrounding soil's nutrients in exchange for fixed carbon. (Bourles et al., 2020).

Studies have shown that AMF cause changes in root morphology and these alteration patterns vary highly depending on plant species. In most cases, AM colonization contributes to higher branching and increased adventitious and lateral roots with a higher proportion of roots of higher orders (Dreyer et al. 2014). In 1992, Hooker et al. observed the branching of second-order and third-order lateral roots of a *Glomus*-inoculated *Populus* population, which produced an increase of 81% in second-order roots and 616% in third-order roots. By significantly increasing the surface area of the plant's roots, the capacity of mineral nutrients and water acquisition increases drastically as did its growth (Bourles et al., 2020). The mycorrhizal fungal mycelia of an infected plant can extend from one plant's roots to others, forming a system known as a common mycorrhizal network (CMN). CMNs can enable multiple plants and even multiple species to transfer nutrients and influence patterns of seedling establishment, interplant competition, plant diversity, and plant community dynamics (Song et al., 2010).

One can think of AMF as an additional barrier between the soil and the cells of the infected host plant. This barrier contributes to the acquisition of crucial moisture and nutrients, it also prevents the acquisition of undesirable articles from the soil whether they be biotic or abiotic. According to a study done by Spagnoletti et al., disease incidence and severity of charcoal root rot caused by *Macrophomina phaseolina* was lower in soybean roots inoculated with *Rhizophagus intraradices* VCh 0011 (2020). Incidence of root rot was reduced by 20% and disease control was 42% by AMF in non-nitrogen-fertilized plants. These results were reflected in a study by Ceustermans et al., where AMF inoculation proved effective in reducing the colonization of nematodes in host plants, nearing reduction degrees of 97% when inoculated with a solution of indigenous AMF species (2018). AMF also caused significant differences in the translocation of metals from roots to shoots in plants co-inoculated and solo-inoculated. Although, metal alleviation in plants inoculated with both *Curtobacterium citreum* and AMF v. AMF only showed no significant difference in metal alleviation, proving that AMF was mainly responsible for metal alleviation. When faced with drought conditions, plants in a symbiotic relationship with AMF showed significantly higher water use efficiency, duration to reach 50% leaf senescence, and duration to survive than control plants, leading them to believe AMF were the cause of the increased water stress tolerance (Boyer et al., 2015).

Hypotheses

		Water Treatment		
		Desiccation	**Regular**	**Abundance**
Arbuscular Mycorrhizal Fungi	**Inoculated**	1	2	3
	Uninoculated	4	5	6

Table 1: Describes how each test group was treated in terms of Mycorrhizal Fungi Inoculation and Water Treatment. Each group is referred to by its group number or Fungi-Water notation. For example, Group 1 is also ID, meaning Inoculated and Desiccated.

This study compared the growth and physiological water tolerances of *Rhiophagus intraradices*-inoculated and uninoculated groups of *Glycine max* of the Envy cultivar. It was hypothesized that mycorrhizal fungi inoculation would increase the growth and chlorophyll content.

Growth Hypotheses:

The null hypothesis states that plant growth (G) will not be different between inoculated and uninoculated sets of soybeans. Groups inoculated with the Mycorrhizal Fungi will not experience higher growth than their similarly water treated, uninoculated counterparts.

H_0: $G_1 = G_4$, $G_2 = G_5$, $G_3 = G_6$ Where: G = average dry mass of living plant

1, 2, 3, 4, 5, 6 = the number correlating to each respective group (Appendix, Table 1)

The alternate hypothesis states that plant growth (G) will be greater in groups inoculated with the Mycorrhizal Fungi than their similarly water treated, uninoculated counterparts.

H_A: $G_1 > G_4$, $G_4 > G_3$, $G_3 > G_6$

Physiological Water Tolerance:

The null hypothesis states that total chlorophyll will not be different between inoculated and uninoculated sets of soybeans. Groups inoculated with the Mycorrhizal Fungi will not experience higher chlorophyll contents than their similarly water treated, uninoculated counterparts.

H_0: $Chl_1 = Chl_4$, $Chl_2 = Chl_5$, $Chl_3 = Chl_6$ Where: Chl = total chlorophyll

1, 2, 3, 4, 5, 6 = the number correlating to each respective group (Appendix, Table 1)

The alternate hypothesis states that chlorophyll content (Chl) will be greater in groups inoculated with the Mycorrhizal Fungi than their similarly water treated, uninoculated counterparts.

H_A: $Chl_1 > Chl_4$, $Chl_2 > Chl_5$, $Chl_3 > Chl_6$

Materials and Methods

Seeds of Glycine max (L.) of the Envy cultivar were scarified in HCl for 30 minutes, breaking down the impermeable membrane surrounding the seeds to expedite the process of germination. After which, the seeds were submerged in distilled water for 24 hours prior to being incubated at 25-28°C in a dark area for 5 days. The germinated seeds were planted in six separate trays of sterilized soil, half of which were treated with a mycorrhizal fungi inoculant containing spores of mycorrhizal fungi *Rhizophagus intraradices.* These two sets of three groups with binary AMF inoculation were known as uninoculated (U, control groups) and inoculated (I). The groups within the two sets received differing levels of water treatment, such as water desiccation (D), regular watering (R), and water abundance (A), each containing 10 plants. The water treatments of each were determined by their percentage of the soil's water holding capacity, calculated by the following equations:

$$Water\ Holding\ Capacity = \left(\frac{fresh\ weight - dry\ weight}{dry\ weight}\right) * 100$$

$$Dessication\ (70\%) = .7 *\ WHC$$

$$Regular\ (100\%) = 1\ *\ WHC$$

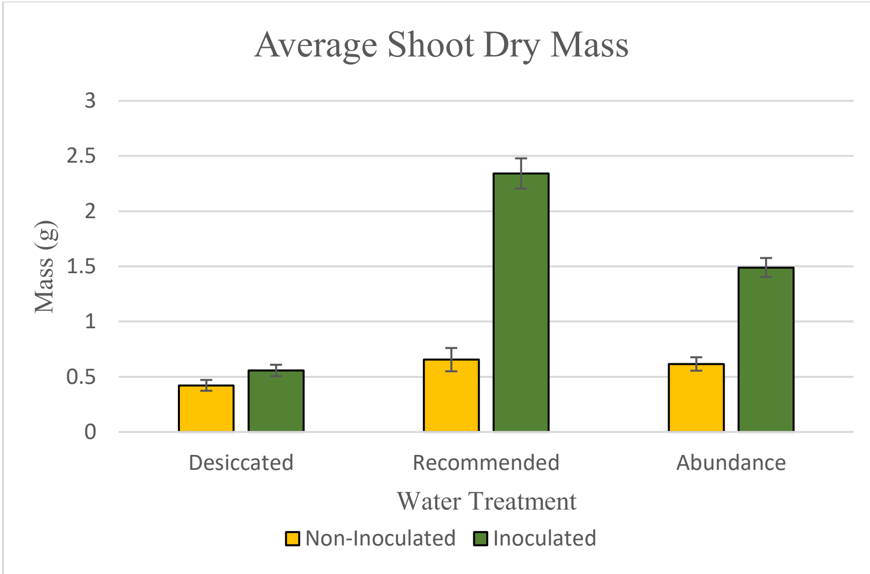
$$Abundance\ (130\%) = 1.3 *\ WHC$$

During a growth period of 2-3 weeks, all experimental groups were watered in such a manner to maintain regular levels of the soil's water holding capacity. This short period was to ensure every group was able to form a baseline of growth for the sake of comparison after the experimental treatments. Following which was a 6-week period of experimental water treatment of 70% water holding capacity, 100% water holding capacity, and 130% water holding capacity, respectively.

After the growth period of 6 weeks, each group was harvested and separated into roots, leaves, shoots, pods, and grains. Roots were cut and washed to remove the soil. All samples were rinsed with distilled water, dried in a forced hot-air oven at 60°C until a constant weight was reached, then measured. Save for the leaves, which were collected for evaluating chlorophyll content. To extract the photosynthetic pigments from the leaves, .25g was randomly collected from each group, then ground in a mortar with 10mL of 80% acetone. After which, these supernatant solutions were brought to a final volume of 25mL using the same 80% acetone and strained to remove any remaining impurities and leaf tissue. The strained pigment solutions were then placed into a centrifuge for 10 minutes, spinning at approximately 3000 rotations per minute in order to quicken the sedimentation of undesired molecules. To quantify the chlorophyllic content of each solution, it was poured into a cuvette and examined at the orange-red light frequencies of 662nm, 652nm, and 645nm using a spectrophotometer. Finally, chlorophyll a and b content were calculated using Wellburn and total chlorophyll was obtained by summing Chl*a* and Chl*b*.

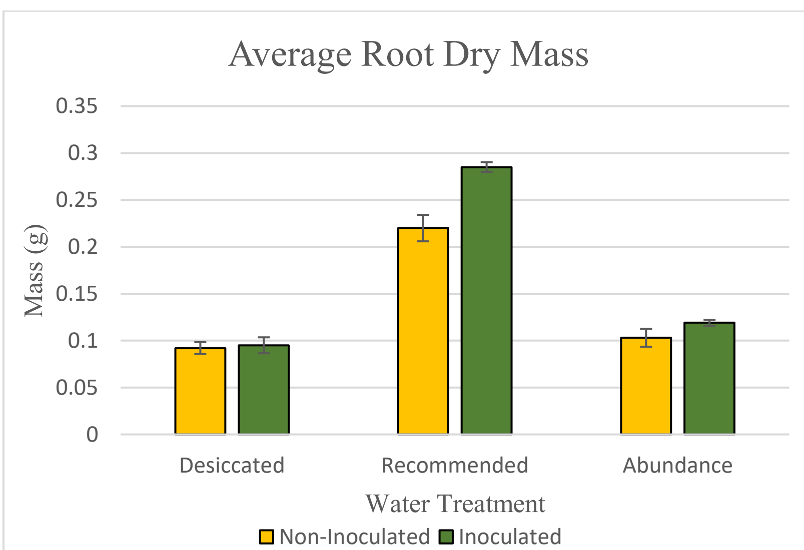
Results

In order to demonstratye differences in mycorrhizal fungi inoculation, the 6 experimental groups were analyzed in terms of average dry shoot mass (Graph 1), average dry root mass (Graph 2), and total chlorophyll content (Graph 3). Although the experiment applied two independent variables, T-tests as opposed to two-factor ANOVAs, were utilized in the calculation of significance. Soybean plants inoculated with the mycorrhizal fungi *Rhizophagus intraradices* significantly increase the dry shoot mass of desiccated groups ($p = 0.007072$), regular groups ($p = 0.011882$), and abundant groups ($p = 0.022543$). Soybeans inoculated with mycorrhizal fungi and received regular water treatment experienced an especially drastic increase in shoot dry mass when compared to the other groups.



Graph 1: This graph represents the average shoot dry mass of experimental groups organized into similar water treatments and color-coded to represent differing mycorrhizal fungi inoculation status. The x-axis shows increasing levels of water holding capacity (from left to right; 70%, 100%, and 130%), the y-axis shows the mass of each average in grams, and the error bars represent one standard deviation within the dataset.

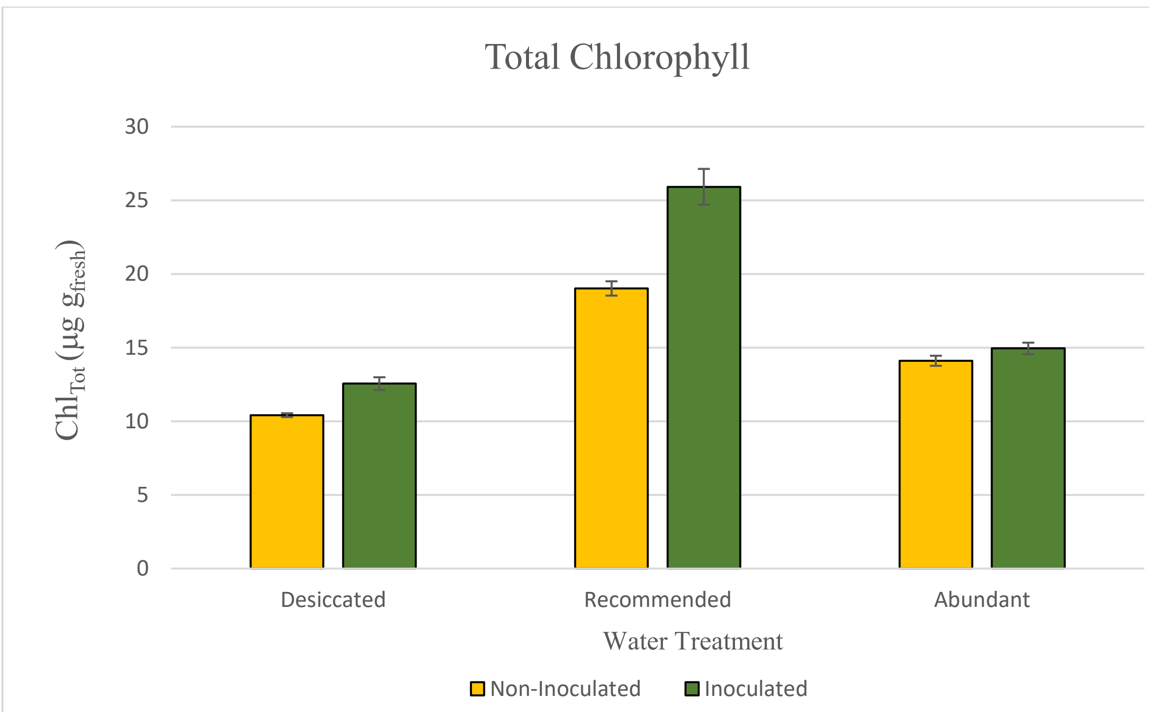
Soybean plants inoculated with the mycorrhizal fungi *Rhizophagus intraradices* significantly increase the dry shoot mass of regular groups (p = 0.024437) but had a much less significant effect on members of the desiccated (p = 0.327979) and abundant groups (p = 0.1142). Soybeans inoculated with mycorrhizal fungi and received regular water treatment again experienced an especially drastic increase in root dry mass when compared to the other groups.



Graph 2: This graph represents the average root dry mass of experimental groups organized into similar water treatments and color-coded to represent differing mycorrhizal fungi inoculation status. The x-axis shows increasing

levels of water holding capacity (from left to right; 70%, 100%, and 130%), the y-axis shows the mass of each average in grams, and the error bars represent one standard deviation within the dataset.

The results and calculations of chlorophyll a, chlorophyll b, and total chlorophyll produced by the utilization of chemistry and spectrophotometry were significant. The mycorrhizal fungi inoculation significantly increased the total chlorophyll content of desiccated groups ($p = 0.031859$), regular groups ($p = 0.02373$), and abundant groups ($p = 0.013704$). Soybeans inoculated with mycorrhizal fungi and received regular water treatment once again experienced an especially drastic increase in chlorophyll content when compared to the other groups.



Graph 3: This graph represents the total chlorophyll content of experimental groups organized into similar water treatments and color-coded to represent differing mycorrhizal fungi inoculation status. The x-axis shows increasing levels of water holding capacity (from left to right; 70%, 100%, and 130%), the y-axis shows the total of each group in micrograms of chlorophyll per gram of fresh leaf, and the error bars represent one standard deviation within the dataset.

Conclusion

This study compares the growth and infection rates of mycorrhizae-infected and mycorrhizae-uninoculated soybean plants in order to better establish the benefits of mycorrhizal fungi-root relationships. It was hypothesized that mycorrhizae-inoculated soybean plants will be more resistant to physiological water stress and experience higher rates of growth than uninoculated plants. To test this hypothesis, 6 experimental groups each representing a permutation of the independent variables, mycorrhizal fungi inoculation and water treatment.

One may conclude from this study that the inoculation of soybeans' root systems with the mycorrhizal fungi *Rhizophagus intraradices* significantly impacts both the growth and physiological tolerance to water stress in the host plant. Significant p-values for shoot dry mass in desiccated groups ($p = 0.007072$), regular ($p = 0.011882$), and abundant groups ($p = 0.022543$) indicated that inoculation likely played a role in the discrepancies between groups. Therefore, the shoot growth null hypothesis may be rejected, and the alternates may be supported. That said, the regularly water-treated groups ($p = 0.024437$) significantly increased in root dry mass when inoculated. However, the non-significant p-values of root dry mass in desiccated ($p = 0.327979$) and abundant groups ($p = 0.1142$) means the null root growth hypotheses fail to be rejected. This is an extremely surprising result, since results from previous studies and observations from this study contribute to the fact that mycorrhizal fungi inoculation has a drastic effect on the density, count, and mass of mycorrhizae-infected host plants. This out of order result most likely surfaced due to a mishandling of or damage to the root samples amidst the process of separation, drying, and massing. The significant p-values (Desiccated $p = 0.031859$, Regular $p = 0.02373$, Abundant $p = 0.013704$) obtained from the total chlorophyll values indicate that for chlorophyll content, the null hypothesis may be rejected, and the alternate hypothesis may be supported.

One possible explanation for the positive effects of mycorrhizal fungi on soybean plants lies in the ability of these fungi to facilitate the uptake of essential nutrients, such as nitrogen and phosphorus, from the soil. By forming a close association with the plant's roots, mycorrhizal fungi can extend the root system's reach, accessing and absorbing nutrients that would otherwise be inaccessible to the plant. This enhanced nutrient uptake can fuel the plant's growth and metabolic processes, leading to greater biomass accumulation and improved overall plant health. In addition to their role in nutrient acquisition, mycorrhizal fungi may also promote soybean plant growth through their effects on root architecture and physiology. Studies have shown that mycorrhizal colonization can lead to increased root length and branching, as well as changes in root cell structure and function. These changes may help the plant to better adapt to stress conditions, such as drought or nutrient deficiency, and to more efficiently absorb and transport nutrients from the soil. Additionally, the impact of mycorrhizal fungi on plant growth and development may stem from their effect on the hormonal equilibrium within plants. Studies show that by colonizing with mycorrhiza, levels of certain hormones in plants - such as auxins and cytokinins which aid cell division and growth - see a rise. This alteration in hormone quantities might be responsible for an increase in soybean plant biomass along with chlorophyll count after exposure to these fungi spores.

Overall, the benefits of mycorrhizal fungi inoculation for soybean plant growth and health are clear. Through their effects on nutrient acquisition, root architecture and physiology, and plant hormone balance, mycorrhizal fungi can enhance soybean plant growth and improve overall crop yield. As such, the use of mycorrhizal inoculants may represent a promising strategy for sustainable and efficient soybean production, with potential benefits for both farmers and the environment.

Sources Cited

Bourles, A., Guentas, L., Charvis, C., Gensous, S., Majorel, C., Crossay, T., Cavaloc, Y., Burtet-Sarramegna, V., Jourand, P., & Amir, H. (2020). Co-inoculation with a bacterium and arbuscular mycorrhizal fungi improves root colonization, plant mineral nutrition, and plant growth of a cyperaceae plant in an ultramafic soil. *Mycorrhiza, 30*(1), 121-131. http://dx.doi.org/10.1007/s00572-019-00929-8

Boyer, L. R., Brain, P., Xu, X., & Jeffries, P. (2015). Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. Mycorrhiza, 25(3), 215-227. http://dx.doi.org/10.1007/s00572-014-0603-6

Ceustermans, A., Wendy, V. H., Jelle, V. C., & Bylemans, D. (2018). Effect of arbuscular mycorrhizal fungi on pratylenchus penetrans infestation in apple seedlings under greenhouse conditions. Pathogens, 7(4), 76. http://dx.doi.org/10.3390/pathogens7040076

Fiorilli, V., Vannini, C., Ortolani, F., Garcia-Seco, D., Chiapello, M., Novero, M., Domingo, G., Terzi, V., Morcia, C., Bagnaresi, P., Moulin, L., Bracale, M., & Bonfante, P. (2018). Omics approaches revealed how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. Scientific Reports (Nature Publisher Group), 8, 1-18. http://dx.doi.org/10.1038/s41598-018-27622-8

Hooker, J. E., Munro, M., & Atkinson, D. (1992). Vesicular-arbuscular mycorrhizal fungi induced alteration in poplar root system morphology. Plant and Soil, 145(2), 207–214. http://www.jstor.org/stable/42938159

Rigamonte, T. A., Pylro, V. S., & Duarte, G. F. (2010). The role of mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations. Brazilian Journal of Microbiology, 41(4), 832-n/a. http://dx.doi.org/10.1590/S1517-83822010000400002

Song, Y. Y., Zeng, R. S., Xu, J. F., Li, J., Shen, X., & Yihdego, W. G. (2010). Interplant communication of tomato plants through underground common mycorrhizal networks. PloS One, 5(10), 1. http://dx.doi.org/10.1371/journal.pone.0013324

Spagnoletti, F. N., Mariano, C., Viviana, C., Lavado, R. S., & Roberts, I. N. (2020). Arbuscular mycorrhiza protects soybean plants against Macrophomina phaseolina even under nitrogen fertilization. European Journal of Plant Pathology, 156(3), 839-849. http://dx.doi.org/10.1007/s10658-020-01934-w

Tominaga, T., Yao, L., Saito, H., & Kaminaka, H. (2022). Conserved and diverse transcriptional reprogramming triggered by the establishment of symbioses in tomato roots forming arum-type and paris-type arbuscular mycorrhizae. Plants, 11(6), 747. http://dx.doi.org/10.3390/plants11060747

Wellburn, A.R. (1994) The Spectral Determination of Chlorophylls a and b, As Well As Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal Plant Physiology, 144, 307-313. http://dx.doi.org/10.1016/S0176-1617(11)81192-2