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Article

# Impact of salinity and phosphorus on growth of *Phaseolus Vulgaris* inoculated with Arbuscular Mycorrhizal Fungi

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## SUMMARY

**As the supply of rock phosphate decreases, it is essential that the agriculture industry reduces wasteful phosphorus (P) fertilizer application by improving crop acquisition of P. Arbuscular mycorrhizal fungi (AMF), a fungus that forms a beneficial relationship with many crops, is one potential solution to this problem. Numerous studies show that crops inoculated with AMF have many benefits such as increased P uptake, resulting in higher yield and resistance to abiotic stresses. The objective of this study was to understand the impact of mycorrhizal symbiosis on bean growth under varying soil conditions of P and salinity. We tested several P concentrations and salinity levels and found that beans planted in soil conditions containing the highest levels of P and salinity exhibited significantly lower root-to-shoot ratios than any other trials while still maintaining similar biomass weight. This suggests that AMF hyphae increased the affinity of the plant for absorbing nutrients, allowing it to focus on shoot growth instead of root growth. We also observed that adding more P fertilizer to the AMF-inoculated bean plants both under salt and non-salt conditions led to diminishing returns in terms of plant height and dry weight. Hence, adding more P fertilizer to compensate for high soil salinity in AMF-inoculated soil could be inefficient and lead to fertilizer run-off that would negatively impact surrounding ecosystems.**

## INTRODUCTION

As the demand for efficient food production rises, scientists expect the use of chemical fertilizers such as phosphorus (P) fertilizers to increase by 51–86% by 2050 (1). This is a problem because P fertilizers can lead to damaging environmental impacts. Natural P in the soil comes from the decomposition of organisms and rocks containing phosphate. Various plants absorb this P and the molecule continues up the food chain. When dead organisms break down, the P is reincorporated into the system which creates a cycle (2). Despite an increase in P fertilizer application, only a small portion of these nutrients are immediately available to crops since around 50% of P in farms is lost due to run-off or soil erosion (3). Moreover, having to extract P from the ground to create fertilizer reduces the amount of P cycling. This lost P ends up in water streams causing an increase in phytoplankton, algae blooms, and eventually eutrophication (4). These effects could lead to potential imbalances in forest and stream ecosystems. Additionally, estimates predict that rock phosphate fertilizer will run out in the next 100 years (5). To protect ecosystems and conserve resources, developing sustainable fertilizer practices is essential. One approach to developing sustainable farming practices is through the use of fungi to increase nutrient uptake.

One type of fungi of interest is arbuscular mycorrhizal fungi (AMF), a type of fungus that forms a relationship with 90% of plant species (6). AMF survives by forming a symbiosis with plants where the plant supplies AMF with sugars and carbon, and AMF increases the nutrient absorption of the host through its hyphae which are fine white strands of cells specialized for their large surface area to volume ratio (7). One problem that plants encounter is that they cannot absorb some forms of organic or inorganic P. AMF indirectly increases plant P absorption by activating local phosphorus-solubilizing bacteria to make the molecule more accessible to plants in the soil (8).

Past studies found that AMF increases overall P uptake in tomato seedlings, mung bean plants, and various citruses, resulting in greater plant growth and yield (9-11). It has also been reported that plants inoculated with AMF have increased uptake of organic P sources, increased resistance to abiotic stresses, which are all non-living factors that negatively influence plant growth, increased pathogen resistance, and stabilized soil structures (7, 12-14). Studies even show that AMF can reduce nutrient leaching and prevent fertilizer run-off (15, 16). While AMF presents many benefits that can be incorporated into sustainable fertilizer practices, its symbiosis with plants is not yet fully understood.

For most crops, P fertilizer (superphosphate) applied before sowing can reduce AMF colonization since the plant has the required nutrients and therefore does not need to form a symbiotic relationship (17). However, it has also been reported that other forms of P fertilizer applied before sowing have negligible impacts on AMF colonization (7). The varying levels of AMF colonization under different types of fertilizer imply that the AMF-plant relationship is complex, and that agricultural workers must optimize soil conditions to effectively utilize fungi in agricultural practices. Previous studies have shown that increased P reduces AMF colonization, but further studies are needed to determine the impact of AMF on plants under conditions containing two or more variables. For instance, researchers found that high salinity levels inhibited AMF hyphal branching and spore

germination thereby reducing chances of AMF colonization (18); however, there is minimal research on AMF symbiosis under two or more abiotic stresses.

Therefore, the objective of this study is to understand the impact of AMF, specifically *Rhizophagus intraradices*, symbiosis on plant growth under varying soil conditions of P and salinity. In this study, we measured plant growth through germination percentage, plant height, fresh weight, and dry weight. The root-to-shoot ratio is also included but is separate from plant growth. It is calculated by dividing root mass by shoot mass. It determines whether plants were focused on growing root structures for nutrient absorption (high root-to-shoot ratio), or whether they were developing shoots for light absorption (low root-to-shoot ratio). Typically, optimal root-to-shoot ratios change depending on nutrient availability, among other factors. The soil conditions consisted of interactions between three P concentrations and two salinity levels. Our hypothesis was that the addition of P alone will increase plant growth and decrease the root-to-shoot ratio of *Phaseolus vulgaris*, also known as the common bean plant. We hypothesize this because the addition of P to AMF-inoculated plants has been shown to decrease colonization in species such as *Petunia hybrida* and *Pisum sativum* (7, 19, 20). Studies have determined that plant roots, under low P condition, release stimulating compounds, such as strigolactones, which act to increase fungal development such as hyphal branching and spore germination (20, 21). It has also been shown that high P conditions meant a lack of or decrease in strigolactone release from plant roots, suggesting that plants could reduce AMF growth in those conditions. Hence we hypothesized that the addition of P alone, while providing bean plants with sufficient nutrients to increase growth, will decrease AMF colonization, decreasing the root-to-shoot ratio as the bean plant would develop its own root structures instead of relying on hyphae. Moreover, we hypothesized that the addition of P and salinity would result in even greater plant growth and decreased root-to-shoot ratios. We observed that an increasing P fertilizer in both salt and non-salt conditions did not result in significant improvement in plant height and dry weight, suggesting that using more P to compensate for high salinity could be unproductive.

## RESULTS

We examined the effect of P fertilizer and soil salinity on AMF-inoculated bean plants by testing, from seven randomly chosen bean plants, the germination percentage, plant height, fresh weight, dry weight, and root-to-shoot ratio for different P and salinity soil conditions. We carried out two trials. The second trial added higher quantities of soil P and salinity with a smaller sample size. Both trials had three levels of P and two levels of salinity for a total of six groups. We included two control soil conditions for both trials: P0 for AMF inoculation with no salt or P, and P0 (no AMF) for no AMF inoculation, salt, or P.

We observed in Trials 1 and 2, visible hyphae growth on the seedlings. After one to three days, some of the seedlings of the non-salt conditions wilted. The hyphae growth continued, reaching the leaves. Taking a leaf sample and placing it under a light microscope revealed that the hyphae matched the description of *R. intraradices*. This overgrowth matches the description of parasitism whereby hyphae wrap around the host plant causing death (22).

### Trial 1

We tested three levels of P and two levels of salinity. We added rock phosphate ($P_2O_5$) for phosphorus treatment. The P concentrations used were 0, 30, 60, and 90 kg P/ha (P0, P30, P60, and P90). The salt quantities used were 0 and 1.4 NaCl g/L (S0 and S1). We included an additional control group of P0 (no AMF) as well. We planted a total of 144 beans per soil condition. To inoculate the beans, we added

**Figure 1: Image showing hyphae growth 14 days after sowing in Trial 1.** There are visible strands of white hyphae, indicating that AMF growth is occurring. This image is representative of all soil conditions in Trial 1 and in Trial 2.

| | | Two-way ANOVA | | |
|---|---|---|---|---|
| | | Salinity Level | Phosphorus Level | Salinity x Phosphorus Level |
| Plant Growth | Germination Percentage | * | n.s. | * |
| | Plant Height 20 Days After Sowing | *** | *** | * |
| | Plant Height 28 Days After Sowing | n.s. | n.s. | n.s. |
| | Fresh Weight | n.s. | n.s. | n.s. |
| | Dry Weight | n.s. | *** | * |
| Root-to-Shoot Ratio | | n.s. | n.s. | *** |

**Table 1: Trial 1 results of two-way ANOVAs on the effects of three phosphorus levels and two salinity levels on germination percentage, plant height after 20 and 28 days, fresh plant weight, dry plant weight, and the root-to-shoot ratio of bean plants.** Orange shaded cells represent significant differences. All the measurements from Trial 1 were inputted into R. ***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. = $p > 0.05$.

*R. intraradices* inoculant into each soil condition. Then, we placed the seeds above the inoculated soil to ensure that roots would make contact with AMF inoculant. We observed hyphae around germinated plants and non-germinated plants 14 days after sowing, indicating that AMF growth was occurring **(Figure 1)**. Over the course of a month, we measured germination percentage, plant height, fresh weight, dry weight, and root-to-shoot ratio. We collected data from the plants a total of six times: germination rate 20 days after sowing; plant height 20 and 28 days after sowing; fresh and dry plant weight as well as root-to-shoot ratio 28 days after sowing.

We collected the germination percentage of each soil condition to determine whether each combination of P and salinity would prevent seedling growth from occurring. Since we performed the experiment once with a large batch of seeds, there were no multiple replicates for the germination percentage. Individually, salinity did have a statistically significant effect on germination percentage ($p < 0.05$, two-way ANOVA) whereas P concentration did not have a statistically significant effect on germination percentage ($p > 0.05$, two-way ANOVA) **(Table 1)**. The germination percentage of salt compared to no salt conditions showed an average of 66% increase in germinated seeds **(Figure 2)**. Overall, increasing the P concentration to P90 did not significantly increase the germination percentage for both the salt and no salt conditions; however, the presence of salinity did increase germination percentage compared to that of non-salt conditions of the same P level. P0 exhibited a lower germination percentage compared to all other experimental groups ($p < 0.05$, Tukey's test).

We measured the height of bean plants 20 days after sowing which revealed that salinity and P concentration, individually, did have a statistically significant effect on plant height ($p < 0.05$, two-way ANOVA). After running Tukey's test for adjusted *p*-values, the plant height from the salt condition of the lowest P concentration was statistically higher than that of the non-salt condition of the lowest P concentration ($p > 0.05$, Tukey's test) **(Figure 3)**. This significant increase suggests that salinity could have directly or indirectly increased the shoot growth of the bean plants. Additionally, we did not observe the same significant difference in higher P conditions, suggesting that adding more P fertilizer mitigated this increased shoot growth in salt conditions **(Figure 3)**.

We collected a second round of data for the plant height 28 days after sowing. There were no significant differences in plant height between all soil conditions ($p > 0.05$, two-way ANOVA) **(Figure 4)**. We did not observe the same significant height difference between salinity levels of the lowest P concentration 28 days after sowing as we did 20 days after sowing.

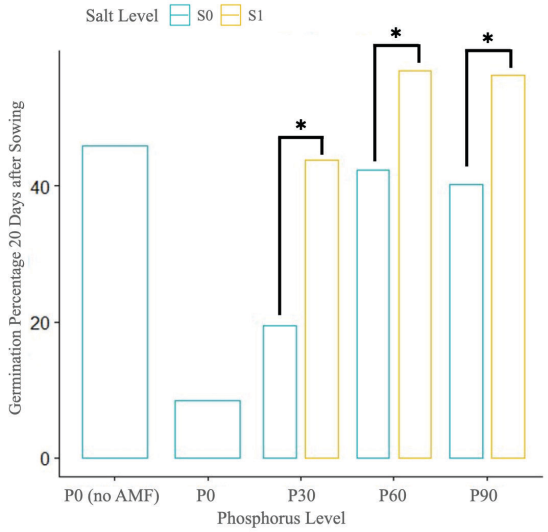


**Figure 2: Germination percentage 20 days after sowing for Trial 1.** The germination percentage of salt conditions were significantly higher compared to non-salt conditions in all three P levels. After 20 days, the number of germinated seedlings were divided by the total number of seedings (144) in each soil condition to obtain the percentage. Asterisks above the bars indicate a significant difference ($p < 0.05$) according to Tukey's post hoc tests (n = 144).

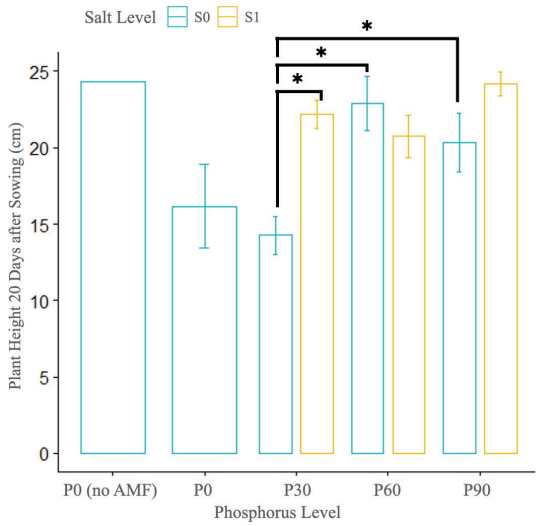


**Figure 3: Plant height 20 days after sowing for Trial 1.** The height of non-salt condition seedlings with the smallest P concentration was significantly lower than that of the corresponding salt condition. The height in S0:P30 was also significantly lower than non-salt conditions in higher P levels. Apart from the lowest P condition, there were no differences in height between salt and non-salt conditions of the same P concentration. The plant height was measured using 16 random samples from each soil condition. Error bars represent ± 1 standard error of the mean and an asterisk above the bars indicate a significant difference ($p < 0.05$) according to Tukey's post hoc tests, (n = 16).

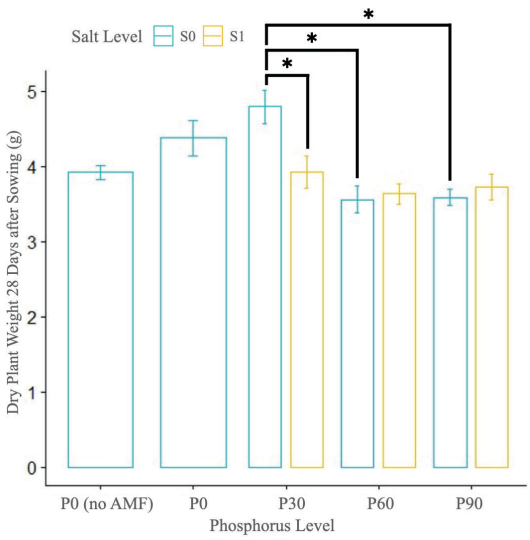


**Figure 5: Dry plant weight 28 days after sowing for Trial 1.** S0:P30 had significantly higher dry plant weight than the corresponding salt condition in the same P level as well as non-salt conditions in higher P levels. There were no differences in weight between salt and non-salt conditions in P60 or P90 conditions. The dry weight was measured by choosing seven random plants from each condition and removing the soil from the roots. The plants were dried overnight at 45 °C and placed on a scale to obtain the dry weight. Error bars represent ± 1 standard error of the mean and an asterisk above bars indicate a significant difference ($p < 0.05$) according to Tukey's post hoc tests, (n = 7).

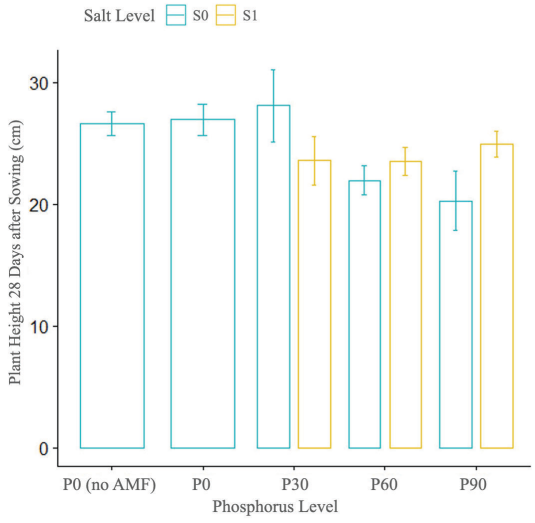


**Figure 4: Plant height 28 days after sowing for Trial 1.** There were no significant differences between phosphorus or salinity groups. The plant height was measured using 16 random samples from each soil condition. Error bars represent ± 1 standard error of the mean and an asterisk above bars indicate a significant difference ($p < 0.05$) according to Tukey's post hoc tests, (n = 16).

We measured dry plant weight to determine biomass changes between soil conditions. 28 days after sowing, we washed the bean plants to remove excess soil and placed them overnight in low heat ovens to remove moisture. The P concentration did have a significant effect on the dry weight ($p < 0.05$, two-way ANOVA). Specifically, in the lowest P concentration, the dry plant weight of the non-salt condition was significantly higher than that of the salt condition ($p < 0.05$, Tukey's test). This difference suggests that the non-salt conditions increased bean biomass production compared to salt conditions in low P concentrations. In addition, the dry weight of salt and non-salt groups of higher P concentrations were not significantly different from each other **(Figure 5)**. Similarly with plant height 20 days after sowing, the significant difference between salinity concentrations in the lowest P group diminished as the P concentration increased.

We measured the plant root-to-shoot ratio to understand whether the bean plants focused on obtaining a higher proportion of roots (to compete for soil nutrients) or a higher proportion of shoots (to increase collection of light). After taking the dry weight, we cut the plants to separate the root from the shoot and weighed them separately to obtain the ratio. The root-to-shoot ratio of the non-salt condition was significantly higher than that of the salt condition in the highest P concentration ($p < 0.05$, Tukey's test) **(Figure 6)**. Additionally, between salt conditions, the beans in the highest P concentration had significantly lower ratios than that of other P concentrations ($p < 0.05$, Tukey's test) **(Figure 6)**.

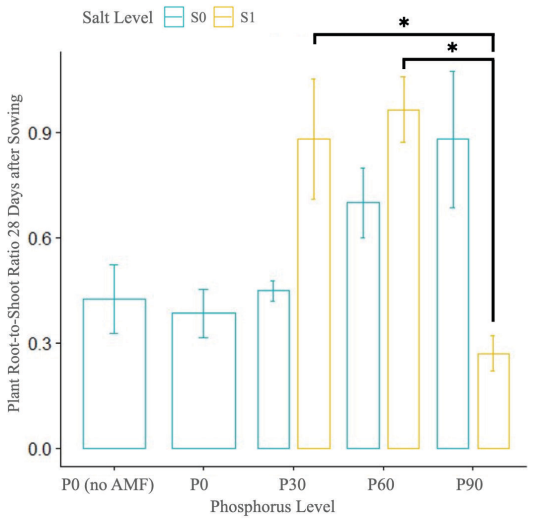


**Figure 6: Root-to-shoot ratio 28 days after sowing for Trial 1.** The root-to-shoot ratio of plants in the highest P and salinity level was significantly lower than the ratio of plants with the same salinity level but smaller P concentrations. The root-to-shoot ratio was measured by choosing seven random plants and drying them overnight at 45 °C. Then, the roots and shoots were weighed, separated, and divided by each other (root mass/shoot mass) to find the ratio. Error bars represent ± 1 standard error of the mean and an asterisk above bars indicate a significant difference ($p < 0.05$) according to Tukey's post hoc tests, (n = 7).

This significant decrease in root-to-shoot ratio observed in high P and salinity levels suggests that some combination of P and salinity caused the bean plants to increase shoot growth relative to root growth. In high P, non-salt conditions, we did not observe the same significant decrease in ratio, thus suggesting a combination of these two abiotic factors is responsible.

### Trial 2

Trial 2 contained higher P and salinity intensities to examine how bean plants would respond in more extreme conditions. For the P concentrations, we used 0, 60, 90, and 200 kg P/ha (P0, P60, P90, and P200). For the salinity levels, we used 0 and 10 NaCl g/L (S0 and S1). The inoculation and planting procedures were identical to Trial 1 except that we planted 12 beans per soil condition. Bean plants in the 10 NaCl g/L conditions had very low germination rates. For the S0:P60 salinity condition, two beans germinated. No beans germinated for the P90 salinity conditions. And one bean germinated for the P200 salinity condition. Because of such low sample sizes, we did not measure bean growth in the salinity conditions. For the non-salt conditions, P concentration did not have a significant effect on plant height 28 days after sowing, fresh weight, dry weight, and root-to-shoot ratio ($p < 0.05$, two-way ANOVA). Based on the non-significant effect, AMF, P, or a combination of both did not significantly affect the observed plant growth under higher P conditions.

## DISCUSSION

The plant height at 28 days after sowing stayed the same as the P concentration increased **(Figure 4)**. This observation is in contrast to previous work that showed that increased P usually results in more plant growth not inoculated with AMF (23). Hence, AMF could have caused this discrepancy in our work. Xie *et al.*, observed a similar behavior in *Kandelia obovata*, where increasing the P application in the AMF treatment group did not increase plant height and in some cases (P 30 mg $kg^{-1}$) led to decreases in height (24). One possible explanation for the decreasing or similar height across groups could be that the increased P acquisition via AMF stimulated root growth rather than shoot growth (which is more commonly observed with nitrogen increase), leading to a decrease in height (25). Another noteworthy observation is that the significant increase in height 20 days after sowing from plants grown in S0:P30 to S0:P60 and S0:P90 disappeared when we measured the plant height eight days later **(Figure 3 and 4)**. A possible reason is that AMF-inoculation in low P conditions, at first, could have slowed shoot growth.

Low levels of P are correlated with high levels of AMF colonization, which suggests that we should see low root-to-shoot ratios for plants grown under S0:P30 conditions **(Figure 5)** (26). This is because under high AMF colonization, the hyphae extend the range of the plant root system for absorbing nutrients, allowing it to focus on shoot growth and decreasing the root-to-shoot ratio. As expected, plants grown under S0:P30 have a low root-to-shoot ratio but a high plant height **(Figures 4 and 5)**. As the P concentration increased in the soil conditions S0:P60 and S0:P90, the plant root-to-shoot ratios increased, suggesting that there was less AMF colonization since the plants needed to form a more developed root structure. This increase in root-to-shoot ratio indicates that the S0:P60 and S0:P90 plants could not focus on shoot growth and therefore had lower plant heights **(Figure 4)**.

In the salinity conditions, the root-to-shoot ratio of plants grown under S1:P90 conditions is roughly three times lower than that of S1:P30 and S1:P60 **(Figure 5)**. The simplest possible explanation for the low root-to-shoot ratio could be that beans sowed in higher P concentrations tend to allocate less biomass to the roots (lower root-to-shoot ratio) because there is already sufficient P acquisition and therefore the bean plant can focus on efficient allocation of resources by increasing shoot growth instead (27-29). Hence, the root-to-shoot ratio of plants grown under S1:P90 conditions could be lower due to the increased P concentration. This explanation appears unlikely because we would expect to have seen a similar decrease from plants grown under S0:P60 to S0:P90. Perhaps the addition of salinity in S1:P90 increased AMF colonization, thereby decreasing the root-to-shoot ratio. We did not measure the exact percent of AMF colonization in this experiment, so it is difficult to determine whether this decreased root-to-shoot ratio was due to high AMF colonization.

Despite a lower root-to-shoot ratio, plants grown under S1:P90 conditions still maintained a similar plant height and plant dry weight compared to other salt conditions. A possible explanation involves AMF; previous research has found that AMF plants with low root-to-shoot ratios can still obtain more biomass than plants with identical root-to-shoot ratios because AMF increases the nutrient absorption of the plant, allowing it to focus on shoot growth (30). This explanation suggests that while plants in the S1:P90 condition had a low root-to-shoot ratio, they could also have high AMF colonization that allowed for similar biomass levels as other conditions. Since high levels of AMF colonization are often observed in low P conditions, we would expect to have seen similar or even lower root-to-shoot ratios for plants grown under S1:P60 and S1:P30 conditions, as those plants would also have high levels of AMF colonization (26). While we did not observe this correlation in our experiment, it has been reported that under salt stress, plants grown in high P concentrations had higher AMF root colonization (31). The addition of salinity combined with P levels could have affected the colonization of AMF. The effect of salinity in combination with other factors on AMF colonization requires further research.

For all measurements except for root-to-shoot ratio, salt conditions show similar values across P concentrations whereas non-salt conditions have significant differences between P concentrations. One possible explanation for this is that AMF and more P under salinity stress may not allow plant growth to exceed a certain threshold. AMF colonization often is negatively correlated with P concentration (7). More P could reduce the beneficial impacts of AMF and create conditions such that the plant cannot absorb the additional P in the soil as efficiently, especially in saline conditions (25). These results suggest that adding excessive P fertilizer to AMF plants to compensate for salinity toxicity would be inefficient.

Looking at the control plants, the dry plant weight of P0 plants showed significantly higher mass than P0 (no AMF) plants, while other metrics such as plant height and root-to-shoot ratio were not significantly different between the two. Studies have also reported increased dry plant weight with plants inoculated with AMF (32, 33). Previous work showed that plants inoculated with AMF have more benefits such as increased nutrient uptake, abiotic stress resistance, and disease resistance (7). These advantages can lead to increased plant growth and in this case, could have resulted in increased biomass. The fact that only the dry weight of AMF-inoculated plants increased compared to P0 (no AMF) could indicate that AMF led to higher root and shoot biomass without increased plant height. Because neither plant height nor root-to-shoot ratio were significantly different between control groups, it is reasonable to conclude that P0 plants had a proportional increase in shoot and root biomass in order to have a higher dry weight, while also maintaining a similar root-to-shoot ratio. Interestingly, P0 had a lower germination percentage compared to other experimental groups. A possible reason for this difference is that the P0 trays were over-soaked since the trays were dipping slightly into the water source below. This could have caused the low germination percentage of P0 plants due to over-soaking.

In Trial 1 and Trial 2, we observed visible hyphae growth on the seedlings. After one to three days, some of the seedlings of the non-salt conditions wilted as hyphae growth reached the leaves. The hyphae taken from these wilted seedlings matched the description of *R. intraradices* and could be parasitism whereby hyphae wrap around the host plant causing death (22).

These seedlings may have already died before the AMF overgrowth. However, dead salt condition seedlings did not have any AMF overgrowth, while most dead non-salt condition seedlings exhibited hyphae overgrowth. A possible explanation for this disparity is that the salinity levels prevented seedlings from developing large enough root structures to form a symbiosis with *R. intraradices* and, instead of wilting due to AMF overgrowth, they wilted due to salinity levels. On the other hand, we expect non-salt seedlings would have formed large enough root structures to develop a symbiosis with the AMF. If the seedlings could not produce enough carbon or sugar for the AMF-plant relationship, this may have prompted hyphae overgrowth due to the lack of nutrients supplied to the fungus.

This explanation does not account for the fact that the majority of non-salt condition plants expressed regular growth and plant height, meaning that only a few plants were affected. According to Smith *et al.*, mycorrhizal symbiosis does not typically lead to diseases under most ecological conditions (7). Since we performed this experiment indoors, it may have caused undue stress to AMF and bean plants which led to hyphae overgrowth. AMF parasitism is possible if the cost of maintaining a mycorrhizal symbiosis exceeds the benefit of maintaining symbiosis for the host plant; under these conditions, AMF will resort to parasitism to ensure its survival instead of allowing the host plant to cut off its carbon supply (34). This behavior is most commonly observed in P-rich, low light conditions, which match the condition of this experiment (35, 36). However, we require more research to determine the specific conditions necessary for AMF parasitism (37).

Two primary limitations in our study are the sample size and the lack of research regarding AMF in complex soil conditions. First, the sample size of this study was a significant limitation. Although Trial 1 produced more germinated bean plants due to the number of seeds used, Trial 2 produced 0-4 plants per soil condition. As a result, our observations and data from Trial 2 did not yield much data on plant growth in higher salinity conditions. Additionally, performing multiple trials instead of sowing all the seeds at once would have allowed for more precise data and more careful handling of plants. Secondly, the overall lack of research regarding AMF and host plant relationships under complex soil conditions also ultimately limits our ability to determine meaningful patterns or connections. As a result, our work serves as an

exploratory study for further research about AMF symbiosis.

This study led to multiple conclusions. We observed that the addition of P fertilizer provided diminishing returns on plant height and dry weight in AMF bean plants under salt and non-salt conditions. The addition of P fertilizers from P30 to P60 reduced the significant differences in height and weight between plants in salinity and non-salinity conditions. In addition, for many of the growth measurements in salt conditions (excluding the root-to-shoot ratio), the P concentrations did not differ significantly, but in the non-salt conditions, they did. The absence of significant plant growth in salinity conditions while increasing P concentrations suggests that using P fertilizer to offset the negative effects of high salinity soils would be ineffective. Moreover, the control plants inoculated with AMF had significantly higher dry plant weight than the plants not inoculated with AMF while having similar root-to-shoot ratios. We could conclude that AMF provided a proportional increase in both shoot and root biomass to obtain a higher weight but similar root-to-shoot ratios. Finally, we observed parasitism among wilted non-salt condition seedlings in Trial 2. According to other studies on AMF parasitism, cases of AMF overgrowth are more common in P-rich, low light conditions which our observations support.

The interactions between AMF and host plants under complex soil conditions are not yet well understood and an important question in the upcoming decade in agricultural ecology is how to modify current practices such as soil management and fertilizer application to improve resource and environmental sustainability without compromising crop output. AMF is already present in almost all ecosystems, including agricultural ones, so learning how to work with it and maximize crop productivity is essential (38). One reason why progress on this front is slow is that different plant species behave differently with various species of AMF, all of which are dependent on the surrounding soil conditions. This complexity shows the importance of studying AMF interactions under two or more abiotic conditions. In order to foster the most efficient symbiotic relationship between soils, AMF species, and plants, scientists must gain a deeper understanding of AMF. If this relationship were to be fine-tuned, AMF-plant interactions could allow for a more efficient and faster intake of P fertilizer, minimizing P application during the growing season.

## MATERIALS AND METHODS

The experiment was conducted in two separate trials. The first trial was conducted using plug trays and the second trial was conducted using vegetation pots. Both trials were done indoors under grow lights (40-watt Sylvania Gro-Lux Wide Spectrum bulbs). The room temperature was 19-21 °C and the humidity was 40%. The plants used were Endeavor Bush beans (*P. vulgaris*, Park Seed); this legume was chosen due to its accessibility and ability to form a symbiosis with AMF. The specific type of AMF used was *R. intraradices* (Xtreme Gardening). The growth medium used was a soilless mix to reduce the chances of soil contamination and soil-borne disease. The soilless mix was made of a three to one ratio of coconut coir (General Hydroponics) to perlite (AeroSoil). The microscope used to observe plant samples was an optical microscope (National Optical).

### Trial 1 – Incorporating phosphorus and salinity

The experiment was conducted using three levels of P quantities and two levels of salinity. Rock phosphate ($P_2O_5$) was used for phosphorus treatment. The P concentrations used were 30, 60, and 90 kg P/ha, labeled in this work as P30, P60, and P90, respectively. The salt quantities used were 0 and 1.4 NaCl g/L, labeled here as S0 and S1, respectively. These values were chosen to be the P concentrations after reviewing similar salinity and phosphorus concentration studies (39, 40). Both P and salt quantities were spread into the soilless mix and then mixed around to ensure even distribution. A control of P0 included AMF inoculation but did not include salt or P. Another control of P0 (no AMF) did not include AMF, salt, or P. These were provided as base measurements to see if any of the soil conditions provided adverse effects on bean growth.

### Trial 1 – Experimental design

A total of eight different soil conditions were tested: P0, P0 (no AMF), S0:P30, S0:P60, S0:P90, S1:P30, S1:P60, and S1:P90. For each soil condition, 144 beans were planted in plug trays. One-inch-deep holes were dug, and 50 mg of AMF inoculant (Xtreme Gardening) was poured into each hole. Afterward, one bean was planted per hole and then covered afterward. Initially, all soil conditions were watered with 1 L of distilled water to add moisture. After that, salinity and P treatments were added according to the specific soil condition. All trays were watered every 1-2 days to retention capacity using distilled water to maintain salinity. The experiment lasted for 28 days after sowing.

### Trial 1 – Measurements

The germination percentage for each soil condition was calculated 20 days after sowing. Plant height was measured twice, at 20 and 28 days after sowing, where 16 plants from each soil condition were chosen at random to be measured. Plant height was calculated from the level of the plug tray to the top of the plant stem. Twenty-eight days after sowing, seven plants were chosen randomly from each soil condition to be measured for the plant dry weight. The soil was removed from the roots. Then, plants were dried overnight in an oven at 45 °C and then weighed to obtain the dry weight. Finally, the roots and shoots of the dried plants were separated using scissors and weighed separately to determine the root-to-shoot ratio for each plant and soil condition (dry weight for the roots/dry weight for the shoot).

### Trial 2 – Incorporating phosphorus and salinity

For the second trial, higher levels of P and salinity were

used to determine the behavior of AMF and bean plants under extreme abiotic stress. The experiment was conducted using three levels of P quantities and two levels of salinity. The P concentrations used were 60, 90, and 200 kg P/ha, labeled here as P60, P90, and P200, respectively. The salt quantities used were 0 and 10 NaCl g/L, labeled here as, S0 and S1, respectively.

### Trial 2 – Experimental design

Similar to Trial 1, eight soil conditions were tested: P0, P0 (no AMF), S0:P60, S0:P90, S0:P200, S1:P60, S1:P90, and S1:P200. However, this time, urea fertilizer was also incorporated into the soilless mix to prevent nitrogen deficiency in plants. For each soil condition, 12 beans were planted in 4-inch pots. The fungi inoculation, P application, and watering methods were identical to Trial 1. For soil conditions containing NaCl, the beans were watered with a salinity of 10 g/L each time. As in Trial 1, the beans were measured 28 days after sowing. The measurements were conducted using methods described in Trial 1.

### Statistical analysis

R software was used to analyze the data. A two-way ANOVA followed by Tukey's test was performed to find significant differences between salinity and P conditions. The data were represented as mean ± one standard error of the mean. All *p*-values less than 0.05 were considered statistically significant.

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