Everybody was Fungus Fighting:

Examining the symbiotic interactions between Ericaceous plants and their beneficial fungal partnerships with Ericoid mycorrhizal fungi and *Trichoderma harzianum*

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Abstract

Mycorrhizal fungi form mutually beneficial partnerships with the roots of nearly all plants. The plants provide carbohydrates to the fungi while the fungi increase the surface area in the network of roots, which increases the absorption efficiency of the plant. Ericaceous plants, such as azaleas and blueberries, associate with a unique type of mycorrhizal fungi that has not been widely studied. RootShield, a biological fungicide product produced by BioWorks, employs another type of beneficial fungi, *Trichoderma harzianum* Rifai strain KRL-AG2, which blocks pathogenic fungi that may cause harm to the plant's roots. It uses enzymes called chitinases to break down the walls of the harmful fungi. This project sought to investigate whether these two varieties of fungi, both of which are beneficial to the plant, can affect each other and ultimately lead to negative consequences when used in combination. While results were inconclusive, this previously unstudied field has a lot of potential research opportunities, which will ultimately provide valuable information for growers of Ericaceous plants.

Keywords: Mycorrhizal fungi, *Trichoderma harzianum,* Ericaceae, azalea, blueberry, RootShield, fungal interactions

Introduction

The importance of plants to human life is widely appreciated, but the effort that plants exert to ensure their own proper nutrition often goes unseen. Plants require sixteen essential nutrients in order to grow properly, some of which are required in large amounts, and others in smaller amounts. Three of these, carbon (C), hydrogen (H), and oxygen (O), compose about 95% of the weight of the plant, and the plant obtains them through air and water. Besides these, the nutrients that plants need the most amount of, called macronutrients, are nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S). Copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), boron (B), molybdenum (Mo) and chlorine (Cl) are considered micronutrients and are required in lower amounts, but are equally as essential for the health of the plant as the macronutrients (Fageria 2007b). These nutrients are taken up by the roots from the soil. Because of these requirements, proper fertilization and nutrient availability is necessary for successful crop and ornamental plant growth to increase yield and commercial value (Fageria, 2007a).

To aid with the retrieval and absorption of vital nutrients, 80% of plants form a mutually beneficial association with mycorrhizal fungi (De Jaeger et al., 2010). As plants transport carbohydrates and other organic molecules through their roots, they leak a portion of these into the surrounding soil, and this leakage is called exudate (Koo et al., 2005). Fungi and other microorganisms gather around the roots to take advantage of the concentration of nutrients. Arbuscular mycorrhizal fungi (AMF), however, out-compete other microorganisms by physically entering into the cells of the roots to obtain carbohydrates in the form of sugar from the plant (Englander, 1980). The plant allows this because in return, the AMF, which is an obligate symbiont, acts as an extension of the root system as it collects water and nutrients from the soil and delivers them to the plant (De Jaeger et al., 2011b). This, in effect, increases the

surface area that the plants have to gather nutrients exponentially. Since the AMF also out-competes other fungi for the root exudates which protects the roots to an extent, this partnership is extremely beneficial to the plant. The vast majority of plants from many different families form these kinds of symbiotic relationships (De Jaeger et al., 2010).

However, AMF are not the only type of fungi that can be beneficial to plants. Fungi from the genus *Trichoderma* are able to grow in a wide range of environments, rapidly colonize the soil, and parasitize a variety of pathogenic fungi. They also are rarely harmful to plants, and their mycoparasitism, a term that describes the relationship of a fungal parasite and its fungus host, can be beneficial to plants who are in peril of pathogenic fungi attack (Nazir Uddin et al., 2018). *Trichoderma harzianum* is a species in this genus, and while it is not an obligate symbiont, it has been shown to form beneficial relationships with plants' roots (De Jaeger et al., 2011b). This fungus shields roots from pathogens such as *Phythium ultimum* and *Phytopthora capsica,* which are microorganisms that can significantly reduce the ability of a plant to transport nutrients, stunting its growth and agricultural production (Nazir Uddin et al., 2018). One variety of *T. harzianum* that is currently on the market is produced by the agricultural supply company BioWorks, which sells *T. harzianum* Rifai strain KRL-AG2 as a biological fungicide under the name RootShield (RootShield WP Biological Fungicide Specimen Label, 2008). According to the BioWorks specimen data sheet, after the product has been applied to seeds it controls plant diseases by growing onto plant roots as they develop. It is also useful for transplants and other propagative material and should be applied prior to the onset of a disease to be the most effective. The company sells RootShield in a variety of forms, including RootShield WP, a wettable powder that can be applied to plants as a drench. After the product has been applied, it will colonize the roots of a plant within 16 to 24 hours and will be an effective method of disease prevention for 10-12 weeks (Francis, 2008).

Using naturally-occurring soil microorganisms such as these two varieties of fungi has become an increasingly popular and reliable source of plant protection and method to boost production. Correct application has the potential to reduce the need for pesticides and fertilizer in agricultural and nursery settings (De Jaeger et al., 2010). However, when AMF is used in conjunction with *T. harzianum* as an inoculum for plants, the interactions between the two fungi can affect how they interact with the plant's root system (De Jaeger et al., 2011b). Contradictory results have been reported. Some researchers have seen that the two types of fungi have not only been able to coexist but seem to mutually improve each other through interaction on the roots of a plant (Amer & Abou-El-Seoud, 2018). Others have seen that the *T. harzianum* mycoparasitizes the AMF but not to an extent that it impedes the AMF's ability to function as an effective symbiont (De Jaeger et al., 2011a). Still others produced data that showed that *T. harzianum* mycoparasitizes the AMF to the extent that the symbiont is less effective at delivering phosphorus to the plant (De Jaeger et al., 2010; De Jaeger et al., 2011b). Some even suggest that AMF has a negative impact on the success of *T. harzianum* (Green et al., 1999).

This project endeavored to study these interactions between *T. harzianum* and mycorrhizal fungi using azalea plants, from the family Ericaceae, as host plants for the fungi. Plants in the Ericaceae family do not have root hairs on their roots. Root hairs are minute extensions of the root epidermis that normally serve to increase the surface area for absorption, and lacking root hairs reduces the plant's ability to absorb nutrients from the soil. Ericaceous plants partially make up for this deficiency by having characteristically dense root systems. Another crucial way that they compensate for their lack of root hairs is by forming symbiotic relationships with Ericoid mycorrhizae, which are a type of mycorrhizal fungi only found in associations with plants in the family Ericaceae. Ericoid mycorrhizal fungi (EMF) also enter into the cortical layer of the root like AMF. This partnership is especially important as it allows the

plants to absorb phosphorus in the soil, which is one of the macronutrients that is normally the most difficult for a plant to obtain in average soil conditions due to its insolubility in soil water (Englander, 1980). Thus, an Ericaceous plant that is hindered from forming these mycorrhizal associations will not be as successful or productive as plants that do have mycorrhizal associations. This feature is exploited in this research in order to evaluate the interference of this symbiotic relationship by *T. harzianum*.

The Ericaceous family contains many species of plants that are important crops. Two examples of these are azaleas, which are a common ornamental plant across the world, and blueberry plants, which represent an important subset of the Florida agricultural market (Debnath, 2008; Popenoe, 2015). Both of these organisms are produced in large numbers, and the cost of using pesticide, both economically and on the environment, on such a large number of plants is significant enough to merit looking into biological control agents to reduce the need for chemical pesticides (De Jaeger et al., 2011a). It would be beneficial to be able to inoculate these plants with RootShield to control fungal pathogens, but not before the effect of the biological fungicide on the EMF fungi has been examined, as the partnership between the plants and EMF is imperative, thus necessitating this research. To my knowledge, the interaction between EMF and *T. harzianum* had not previously been studied.

Materials and Methods

To conduct my study, I grew azalea plants from cuttings, based on the protocol outlined in the University of Florida IFAS extension Azalea Fact Sheet (Popenoe, 2015). At the end of the summer, after the bushes had flowered and grown new shoots from the junction where the flower fell off, I took fresh cuttings off healthy established plants from the Florida Southern College campus. I wounded the ends, dipped them in the rooting hormone Hormodin 3, inserted the

cuttings into individual plastic 4-inch pots, and allowed them to root in a mist bed for 11 weeks. By growing the experimental specimen from cuttings, I was able to accelerate the time it took them to mature as opposed to growing the plants from seeds while being able to control the exposure of the plants to the fungi since they grew an entirely new rhizosphere. I rooted 180 cuttings, but unfortunately, I did not have the resources available to me to take all of the cuttings from the same mature individual so the cuttings were from a variety of bushes growing in the same area that flowered different colors. The mist bed where the plants were housed as they rooted was outside and exposed to the elements, but it ensured that all cuttings stayed consistently moist, and any weather conditions were experienced evenly across all of the cuttings so should not be a confounding factor to the data. The soil used was Sungro Sunshine Mix #8/ Fafard-2 Professional Growing mix. I measured the pH of the soil to be 5.25, which is in the ideal range for azaleas.

To inoculate the plants with fungi, I used RootShield WP as the *T. harzianum* source, and an inoculant of mycorrhizal fungi from the roots of the azaleas growing on Florida Southern College campus. I had four experimental sets of 45 plants each; one set of plants was exposed to EMF only, one set to Root Shield only, another set to both types of fungi, and a final set was not inoculated with either fungus. The plants were exposed to the fungi in week 7 of their growth, while they were still rooting in the mist bed but did have some initial root growth. I exposed them at this time so that the young roots were exposed to the fungi as soon as they appeared. There was no previous protocol established for the inoculation of plants with EMF because the method that is often used for growing AMF for use as an inoculant is not possible for EMF. Since mycorrhizal fungi is an obligate symbiont, it must have a host plant in order to successfully propagate throughout soil, and since EMF has exclusive partnerships with Ericaceous plants, a quick-growing host plant like grass cannot be used to grow inoculant (De

Jaeger et al., 2011b). Therefore, I created my own method for EMF inoculation. I collected samples of EMF from the soil around the same mature azalea plants that I took the cuttings from since the fungus is visible on the roots of the Ericoid plants. Then, I removed dirt from the samples by rinsing them with water and removed as much woody matter as was possible. I then divided the fungi into pieces that weighed between .5 grams and .9 grams when dry (see *Figure 1A* for exact measurements), poked a 1-inch-deep hole in the soil directly next to the stem of the cutting using a pencil, inserted the fungi, and closed the hole with soil. For the plants that were exposed to RootShield, I mixed 1 tsp of the wettable powder with 2.5 gallons of water, which is the concentration recommendation according to the packaging. I poured 100 ml of the mixture into the soil of the cuttings, which I did in stages to allow the liquid to absorb fully into the soil before pouring the rest of the 100 ml. To avoid the possibility of the Root Shield fungi contaminating the soil of the cuttings that were not to be inoculated with it, I made sure that any excess liquid had drained out of the pots before placing them near the other cuttings. For the set of plants that were exposed to both EMF and RootShield, I completed both of the processes described above, and the mass of EMF applied to those cuttings can also be seen in *Table 1A*.

Within each of the four groups of plants, I had three subsets of 15 plants each. One subset was not given any fertilizer, one subset was given quarter-strength fertilizer, and the final subset was given half-strength fertilizer. I did not give any plants full-strength fertilizer since the effects of mycorrhizal fungi can be seen best when the plant is stressed out, as the EMF helps to retrieve nutrients more efficiently than bare roots (De Jaeger et al., 2011a). I performed this in order to be able to best observe the effect of the AMF and to be able to examine if the associations with AMF were stronger on the plants that were more stressed for nutrients than those who had more nutrients readily available to them. I used Miracle Gro Water Soluble Azalea-Camellia-Rhododendron Plant Food to fertilize the plants since azaleas are acid-loving plants. I applied

this fertilizer on Week 9 of the cuttings' growth, 2 weeks after they had been inoculated with fungi, and 2 weeks before removing them from the mist bed. This timing was chosen to allow the plants to establish connections with the fungi before having supplies of nutrients available, while also creating the experimental conditions for each plant as early as possible so as to be able to differentiate the impact of the fungal partnerships for each condition. For each subset, I applied 100 mL of the fertilizer solution to the cuttings, and for the subsets that did not receive fertilizer, I poured 100 mL of water alone to negate any differences that the additional liquid may have led to. For amounts of fertilizer for each subset, see *Table 2A*.

To grow the azalea cuttings, both while they were in the mist bed, and after they were removed and placed in a greenhouse area with bright, indirect light, I grouped the different experimental conditions in a randomized block design. I rotated the position of the pots in their blocks biweekly to ensure that sun or other environmental effects did not skew the data, as advised by Fageria (2007a). I also kept a gap of about 6 inches on the benches in between the cuttings with different fungi to avoid contamination between the experimental sets. On a biweekly basis, I collected data on the azalea cuttings, which consisted of checking if they were rooted, recording if they were budding or flowering, measuring their height, and recording their color using the Munsell Color Charts for Plant Tissues (Munsell Color, 2016). In order to consistently measure the height of the plants across all of the data sets, I used a hard-plastic ruler, placed it firmly on the soil next to the plant, and measured to the highest point on the plant without bending any horizontal or leaning branches up. I measured to the top of the highest stem, not leaf. Additionally, every time I measured the plants I removed any detritus that had collected in the pots during the 2-week interval, in order to eliminate any nutrient imbalances that could arise between different individuals due to the degradation of fallen leaves or flowers. Figures to show some of the details of the measurement process can be found in Appendix B.

Results

Due to the untimely interruption of the novel Coronavirus (COVID-19), I did not get to collect all of the data that I had intended. While I collected data on the growth rate and coloration of the youngest leaves of each cutting, I was unable to dig the plants up at the end to examine their root structure or how established the fungal partnerships were. The average change in height for each set and subset can be seen in *Table 3A*, and the average growth for cuttings inoculated with EMF, both with and without RootShield, is lower than or equal to the least amount of growth for either of the other two conditions. Cuttings inoculated with RootShield showed the most amount of growth, with an average change in growth of 3.34 cm compared to 2.28 cm for the control cuttings with no fungi, 1.60 for the cuttings with EMF only, and 1.70 for the cuttings with both EMF and RootShield. Over the course of the experiment, 20.6% of the cuttings died, and the percentage of mortality in each experimental data set is found in *Table 4A*.

The Munsell Plant Tissue color data that I collected throughout the semester, in general, showed several trends across all of the plants. The first trend is that all of the plants began to show paler, more yellow leaves beginning after their first week in the mist bed, and continuing to become paler and more yellow until at least their 7^{th} or 9^{th} week in the mist bed. Then, the plants seemed to mostly plateau and continue with the same tissue color until the end of the 25^{th} week, when the last measurement occurred. However, some plants did begin to recover their darker pigments toward the end of the time period, beginning around the 17^{th} or 19^{th} week of growth. These trends seemed to be applied randomly, and did not happen in any specific experimental condition, but rather across all of the plants (data not shown).

Discussion

The results of this experiment are not conclusive. This is due to several factors. Primarily, my timeframe was truncated, and this experiment should be carried out over a longer period in the future in order to have more time to observe the growth and health of the plants because the growth of plants is time-consuming and cannot be sped up. Secondly, my data sets were too small which was due to limited resources and manpower. Having more plants to analyze would make the differences between growth rates more obvious and less likely to be due to random coincidences. For instance, while there is a significant difference between the growth of the plants that were exposed to RootShield only and those that were exposed to EMF or no fungi at all (*Table 3A*), it is not consistent enough across all of the plants in the set to be able to conclusively say that the RootShield definitively accelerated the growth of the plants. With a larger data set, that difference may be more consistently obvious and allow for stronger conclusions. The same goes for the death rates in *Table 4A*. While the plants with RootShield only had the highest rate of death of the four experimental conditions, the numbers are not large enough to show that these results have significance. This is again illustrated in the growth data in regards to the level of fertilizer provided to each subset within the four data sets. There is no consistent pattern showing that the greater amounts of fertilizer aided in faster growth, and while this could be due to the fungal interactions on the roots of the soil, the differences are too small to discern and the number of 15 individuals per subset is much too small to base conclusions on.

Another reason I cannot draw conclusions about the fungal interactions with the plant and which partnerships were most beneficial is because the only data that I was able to collect was the preliminary growth rate and color change data. In order to more accurately study the plants, I would need to be able to dig up the plants to examine their root systems, since throughout the experiment I could only see half of the plant. Plants in the family Ericaceae have characteristically dense root systems, and examining their health, size, and any evidence of

fungal interactions would be crucial to determining the consequence of the results (Englander, 1980). Additionally, since I used a novel method of inoculation with EMF and was unable to dig up the plants to examine the roots, I was not able to verify whether the inoculation technique is effective.

In regards to the tissue color data of the plants, the trend towards paler, more yellow leaves is most likely due to nutrient deficiency, which is logical because at the beginning, right after I planted the cuttings, they did not have roots and had no way of obtaining nutrients from the soil (Englander, 1980). They gradually lost color as they used the stored resources available to them for energy. Once the azaleas grew roots, the plants were able to pause and, in some cases, reverse the effects and begin to obtain nutrients from the soil. The fact that the color trend did not vary reliably from one experimental condition to another seems to indicate that this trend would occur no matter the circumstances of the cuttings and that the fungal interactions or nutrient level in the soil did not have a significant impact. If the experiment had been allowed to continue, the differences in color recovery in the leaves may have become more obvious, as the plants with the most efficient roots and the highest levels of nutrients from the fertilizer would be best able to recover the nutrients lost to them in their rooting process.

The high mortality rate of the azaleas throughout the experiment, with a total of 37 plants out of 180 dead by the end of the 25th week since being planted, could be due to differing quality of the cuttings. Since I had to take cuttings from several different azalea bushes, it is possible that one bush was in poorer health or yielded less viable cuttings than others. As previously mentioned, I cannot draw conclusions as to the quality of the experimental conditions based on the mortality rate data since the data sets were not sufficiently large.

In the future, there are many potential areas of research to investigate the interactions between RootShield and EMF. This initial research that I attempted should be done again, on a

larger scale and with a longer time period. Developing a successful way to inoculate plants with EMF, obtaining more conclusive results from the growth rate and tissue color data, and being able to examine the root structures of the plants and compare the health of plants from each of the four experimental conditions are important first steps in this field of research. Additionally, it may be beneficial to further study whether the order in which the plants are exposed to each fungus has any bearing on their health. Another way to strengthen the quality of the results obtained would be to ensure that all the cuttings are from the same mature azalea plant, or at least from the same species living in very similar conditions. Furthermore, it will also be important in the future to conduct similar experiments in a completely sterile environment using tissue culture in order to be able to have complete control over the exposure of the plants to the fungi, as contamination by fungal spores is very likely in any non-sterile environment and most likely occurred in my research. Experimenting in a sterile environment would provide results that can be compared with those of the non-sterile experiment, since any large discrepancies between the results may reveal that there was contamination or other confounding factors that affected the results of the non-sterile experiment (Debnath, 2008). After those experiments have been compared, fieldwork should also be done, and experiments should be conducted in conditions as close as possible to those of commercial azalea and blueberry plants, in order to ensure that any conclusions drawn from this research are applicable and realistic for commercial growers or plants in the Ericaceous family.

Originally, I set out to execute this experiment in order to begin research on the previously unexamined interactions between Ericoid mycorrhizal fungi and *Trichoderma harzianum*, both of which are beneficial fungi to plants. While unfortunately the original goal of this research was rudely interrupted by the global pandemic, I hope this project instead serves to bring attention to the topic, as these unstudied fungal relationships have a great potential for a

multitude of applications. From blueberries and cranberries to azaleas and rhododendrons, the Ericaceae family of plants is widespread and both economically and ecologically valuable (Fageria, 2007a). Studying how RootShield can be implemented in commercial applications to help reduce disease while ensuring that it does not interfere with the plants' important symbiotic relationship with EMF, may reveal valuable information and ensure that growers either do not waste money on unnecessary pesticides when they could be using a biological fungicide, or ensure that they do not unknowingly harm their plants by inducing an underground war between fungi.

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Appendix A

Plant Number	Mass of EMF (g)	Plant Number	Mass of EMF (g)	Plant Number	Mass of EMF (g)	Plant Number	Mass of EMF (g)	Plant Number	Mass of EMF (g)
MF 46-	0.7 g	MF64+/-	0.8	MF82+	0.5	MFRS145-	0.8	MFRS163+/-	0.6
MF 47-	Previously Deceased	MF65+/-	0.7	MF83+	0.6	MFRS146-	0.6	MFRS164+/-	0.5
MF48-	0.6	MF66+/-	0.7	MF84+	0.6	MFRS147-	0.6	MFRS165+/-	0.7
MF49-	0.5	MF67+/-	0.6	MF85+	0.5	MFRS148-	0.7	MFRS166+	0.6
MF50-	0.6	MF68+/-	0.5	MF86+	0.5	MFRS149-	Previously Deceased	MFRS167+	0.8
MF51-	0.5	MF69+/-	0.5	MF87+	0.7	MFRS150-	0.7	MFRS168+	0.6
MF52-	0.5	MF70+/-	0.5	MF88+	0.5	MFRS151+/-	0.5	MFRS169+	0.7
MF53-	0.7	MF71+/-	0.9	MF89+	0.6	MFRS152+/-	0.8	MFRS170+	0.5
MF54-	0.7	MF72+/-	0.9	MF90+	0.5	MFRS153+/-	0.5	MFRS171+	0.7
MF55-	0.8	MF73+/-	0.7	MFRS136-	0.5	MFRS154+/-	0.5	MFRS172+	0.7
MF56-	0.6	MF74+/-	0.6	MFRS137-	0.8	MFRS155+/-	0.6	MFRS173+	0.7
MF57-	0.6	MF75+/-	0.9	MFRS138-	0.8	MFRS156+/-	0.6	MFRS174+	0.7
MF58-	0.8	MF76+	0.6	MFRS139-	0.5	MFRS157+/-	0.9	MFRS175+	0.5
MF59-	0.6	MF77+	0.7	MFRS140-	0.5	MFRS158+/-	0.5	MFRS176+	0.7
MF60-	0.6	MF78+	0.5	MFRS141-	0.6	MFRS159+/-	0.5	MFRS177+	0.6
MF61+/-	0.8	MF79+	0.5	MFRS142-	0.7	MFRS160+/-	0.7	MFRS178+	0.5
MF62+/-	0.8	MF80+	0.7	MFRS143-	0.9	MFRS161+/-	0.5	MFRS179+	0.7
MF63+/-	0.5	MF81+	0.6	MFRS144-	0.8	MFRS162+/-	0.8	MFRS180+	0.6

Table 1: Mass of the EMF inoculant that was inserted into the soil of each cutting, where MF refers to the cuttings that were only exposed to mycorrhizal fungi, and MFRS refers to the cuttings that were exposed to mycorrhizal fungi and RootShield.

Subset	Fertilizer Rate	Subset	Fertilizer Rate
C-	100 mL water only	RS-	100 mL water only
C+/-	100 mL of solution at quarter strength (1/4 tbsp per gallon)	RS+/-	100 mL of solution at quarter strength (1/4 tbsp per gallon)
C+	100 mL of solution at half strength (1/2 tbsp per gallon)	RS+	100 mL of solution at half strength (1/2 tbsp per gallon)
MF-	100 mL water only	MFRS-	100 mL water only
MF+/-	100 mL of solution at quarter strength (1/4 tbsp per gallon)	MFRS+/-	100 mL of solution at quarter strength (1/4 tbsp per gallon)
MF+	100 mL of solution at half strength (1/2 tbsp per gallon)	MFRS+	100 mL of solution at half strength (1/2 tbsp per gallon)

Table 2: Rates of fertilizer application, where the sets are C for control (no fungi), MF for mycorrhizal fungi, RS for RootShield, and MFRS for mycorrhizal fungi and RootShield; the subsets are – for no fertilizer, +/- for quarter strength fertilizer, and + for half strength fertilizer.

Subset	Average Change in Height (cm)	Subset	Average Change in Height (cm)	Set	Average Change in Height (cm)
C-	2.12	RS-	2.00	C	2.28
C+/-	2.80	RS+/-	3.81	MF	1.60
C+	1.90	RS+	4.20	RS	3.34
MF-	1.00	MFRS-	1.70	MFRS	1.70
MF+/-	1.80	MFRS+/-	1.60		
MF+	1.80	MFRS+	1.90		

Table 3: Average growth in cm of the cuttings in each subset over a period of 25 weeks, where the sets are C for control (no fungi), MF for mycorrhizal fungi, RS for RootShield, and MFRS for mycorrhizal fungi and RootShield; the subsets are – for no fertilizer, +/- for quarter strength fertilizer, and + for half strength fertilizer.

Subset	Number of dead cuttings at the end of 25 weeks	Percent of total	Subset	Number of dead cuttings at the end of 25 weeks	Percent of total	Set	Number of dead cuttings at the end of 25 weeks	Percent of total
C-	2	13.3%	RS-	4	26.7%	C	8	17.8%
C+/-	5	33.3%	RS+/-	3	20.0%	MF	8	17.8%
C+	1	6.7%	RS+	5	33.3%	RS	12	26.7%
MF-	2	13.3%	MFRS-	2	13.3%	MFRS	9	20.0%
MF+/-	3	20.0%	MFRS+/-	6	40.0%	Total Deaths	37	20.6%
MF+	3	20.0%	MFRS+	1	6.7%			

Table 4: Rate of death for each of the sets and subsets for a period of 25 weeks.

Appendix B

Figures 1 and 2: Ericoid mycorrhizal fungi on the root dug up from a mature azalea plant on the Florida Southern College campus. The slightly yellowish protrusions are the hyphae of the fungus. The first picture is as seen with the bare eye, and the second is through a dissecting scope at 11X.

Figure 3: EMF in preparation for inoculation after being divided. The EMF was allowed to dry before being weighed.

Figures 4 and 5: Azalea cuttings about to be measured