JOURNAL OF UNDERGRADUATE RESEARCH

Mycorrhizae Quantification in Sapelo Island Sand Dunes

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Abstract

Mycorrhizae have played a vital role in plant growth for over millions of years. This fungus forms a symbiotic relationship with the plant roots that stimulate plant growth and root development. This symbiotic relationship suffers in ecosystems, which have been disturbed by human activity resulting in a decline of mycorrhizae. Many journals have been published in accordance to mycorrhizae research, but a knowledge gap has been formed due to the lack of publishing in the 21st century. In the present article, the researcher developed an approach to further understand the concentration of mycorrhizae throughout a sand dune and what effect that has on the community. The motivation behind this research is to gain a better understanding of mycorrhizae's relative importance in coastal dune systems, especially their role in helping these systems recover from disturbances, such as hurricanes. This review studies closely the quantification of mycorrhizal infection in Uniola paniculate (sea oat) and Spartina alterniflora (cord grass) plants found in sand dunes on Sapelo Island, Georgia. This study focuses on two types of plants, with 30 plots from three different sections of the sand dune; beachfront, midlevel, and upper level in the presence of wrack. All roots went through a staining process in the lab in order to visually quantify amounts of vesicles, arbuscules and hyphae. From laboratory observation it is evident there are high levels of infection throughout the plots. Thus, this particular sand dune has relatively healthy plants with high nutrient and water availability despite being exposed to hurricanes.

Keywords: Mycorrhizae, arbuscules, hyphae, vesicles

Introduction

Mycorrhizae are an ancient fungus that have played a symbiotic role in plants for hundreds of millions of years (Remy, et al. 1994). There are many types of mycorrhizae, but arbuscular mycorrhizae (AM) are the most common type and are the type of mycorrhizae this paper will focus on (Smith & Read, 2008). As stated previously, the fungi to host relationship is mutually beneficial. Excess sugar that is produced in the plant's leaves get sent to its roots where the mycorrhizae absorb the sugar (VanSomeren, 2016). Since mycorrhizae are located underground, they rely on the plant's photosynthesis since they do not see any sunlight. In return, the fungi draw in water and nutrients from the surrounding soil that the host's root system would not be able to accomplish on its own. In addition to helping with the host's nutrient availability, mycorrhizae make the host plant less susceptible to pathogens found commonly in the surrounding soil as well as improve the plant's drought and salinity tolerance (Berruti, et al.

2015). This symbiotic relationship is found in the roots of more than 80% of land plants (Smith & Read, 2008). However, in soil that has been exposed to anthropogenic or natural disturbances, the concentration of mycorrhizae in plant roots decreases. The motivation behind this research is to gain a better understanding of mycorrhizae's relative importance in coastal dune systems, especially their role in helping these systems recover from disturbances, such as hurricanes. To explore this, the researchers surveyed mycorrhizal structures – hyphae, arbuscules, and vesicles, in the roots of two types of dominant dune building plants, *Uniola paniculate* (sea oats) and *Spartina alterniflora* (cord grass).

Hyphae are the first to from once a host plant is infected. Hyphae interconnect the two environments; the roots and surrounding soil (Jansa & Bukovska, 2013). They act as a pathway for nutrients and water to go from the soil, to the mycorrhizae, then to the plant. Like mycorrhizae, hyphae cannot be seen by the naked eye. However, mycelium are connections of hyphae that are so abundant they can be seen without a microscope (Powell, 1984). The hyphal branching then goes into the plant cells and starts forming arbuscules (Marx, 1999). Once the arbuscules are formed, they become the site for fungi to plant metabolic exchange (Powell, 1984). Arbuscules then begin to branch of dichotomously and create smaller branches. Arbuscules take several days to form, and their average life span is only 4 to 5 days then their branches begin to deteriorate (Powell, 1984). After the arbuscules degrade, vesicles begin to from. At the beginning of their life, vesicles contain nuclei, granules and small vacuoles, but once they are mature, they contain large lipid droplets (Powell, 1984). Because of these large lipid droplets, vesicles play a vital role in storage and carbohydrate uptake (Marx,1999). Interestingly, in dead root sections, vesicles can reinfect and regrow hyphae in order to infect new roots (Smith & Read, 2008).

This paper will quantify the amount of arbuscules, vesicles and hyphae found in different plots from a sand dune on Sapelo Island, Georgia. It is hypothesized that larger more stable dunes found higher on the bank may have higher levels of mycorrhizal infection, since they are farther from anthropogenic disturbances and are better suited to withstand harsh hurricanes.

Methods and Materials

In November of 2019, plant roots were acquired from different plots in a sand dune on Sapelo Island, Georgia. For each plot, the sand was dug up until the roots were exposed. The most abundant roots occurred at a depth of approximately six inches from the top soil. For each plot, several roots were taken to ensure extra samples would be available in case replicates needed to be done in the lab. Once the roots were extracted from the ground, there were stored in a cooler with ice until they were back in the lab the next day. Once in the lab, the roots were stored in 50% ethanol in order for the roots to remain preserved. The roots were stored in the ethanol at room temperature for approximately three weeks.

Next, 10 one-inch root samples from each plot were placed into individual cassettes. The 10 root samples were wrapped in a thin permeable cloth, placed in a cassette, and wrapped with a rubber band to ensure stabilization. The cassettes were submerged into a solution of 2.5% KOH and were placed in a water bath at 90 degrees Celsius for 25 minutes. Next, the cassettes were rinsed thoroughly in water. Afterwards, the roots were soaked in alkaline hydrogen peroxide for 20 minutes. Roots were then rinsed with water and submerged in 20-50 vol. 1% HCl for 15.5 hours. Cassettes were taken out of the solution and rinsed immediately. A dye solution was made from 1% ink and 25% acetic acid. This solution was used to stain the roots instead of using trypan blue, as suggested by Phillips and Hayman (1970). The trypan blue used in the past procedures has been listed by the International Agency for Research on Cancer as a possible carcinogen (Vierheilig, et al. 1998). Hence, an ink and vinegar solution was used in its place as suggested by Vierheilig and Coughlan (1998). Once roots were submerged in the ink and vinegar solution, they were placed in a water bath at 90 degrees Celsius for 20 minutes. Afterwards, the cassettes were rinsed with water and stored in a refrigerator.

The next step was to quantify the number of vesicles, arbuscules, and hyphae. From each plot, eight one-inch roots were examined under a microscope. The roots were cut open with a scalpel to expose the inside and outside of the root. Once placed under the microscope, the root was looked at in sections. In each section, it was noted if there was the presence of vesicles, arbuscules or hyphae by marking a “1” if they were seen and a “0” if they were absent.

All samples for this experiment were collected from a sand dune on Sapelo island, Georgia. The location of this island can be seen in Figure 1. This specific location was chosen because it is a dune system that has been exposed to two recent hurricanes.

Figure 1. Map showing where Sapelo Island is located
"Map of Sapelo Island, Georgia." *Live Beaches*, 2020.

Results

This experiment surveyed the roots of two plants, as seen below in Figure 2 and Figure 3. These two plants were distributed throughout four plots labeled: EMB, Rich, Sea Oat, and SPAP. Plots labelled "Sea Oat" are the pioneer plants growing on the beach front and "SPAP" plots refer to the spartina plants that were also growing on the beach front. Plots labeled as "Rich" are the sea oat plants that were growing on the dune ridge and "EMB" stands for embryonic dune patches.

To quantify the proportion of vesicles, arbuscules and hyphae found in each plot, the percentage of roots that were colonized were found. That is why, in the graphs below, the y-axis is a proportion that goes from zero to one.

Figure 2. An image of Uniola paniculate (sea oats)
R. Alan Shadow USDA NRCS East Texas Plant Material Center

Figure 3. An image of spartina alterniflora
USDA NRCS National Plant Materials Center Beltsville, MD.

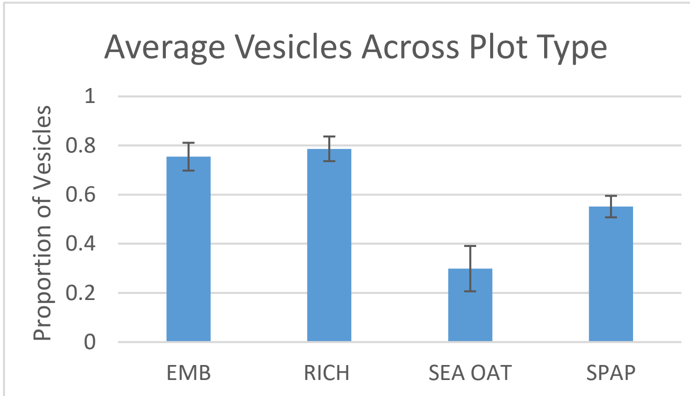

Figure 4. Illustration showing the average proportion of vesicles across each plot type

Figure 4 depicts the average proportion of vesicles across all four plot types. By looking at the graph, it is evident the plots from “EMB” and “RICH” had the highest level of vesicles infecting their roots, with about 75% of these plots’ roots containing vesicles. The sea oat plants that were growing on the beach front had the least amount of vesicles found in their roots. The primary role vesicles play is for storage; however, vesicles are also responsible for carbohydrate uptake from the host roots. Figure 5 shows vesicles under a microscope.

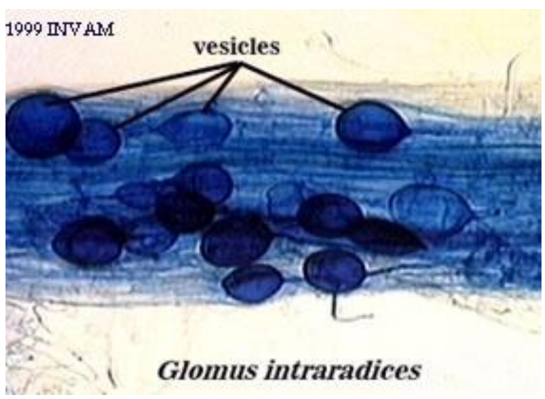

Figure 5. Microscopic image showing the globose bodies caused by swelling of hypha which are known as vesicles. (Marx 1999)

Next, the proportion of arbuscules was examined across each plot type, as seen below in Figure 6. When comparing Figure 4 and Figure 6, it is evident the number of roots infected by arbuscules was much lower. Where in Figure 4, the highest infection proportion was just under 80%, in Figure 6, the highest infection proportion is approximately 27% meaning the roots contained much higher levels of vesicles than arbuscules. Figure 7 shows arbuscules underneath a microscope.

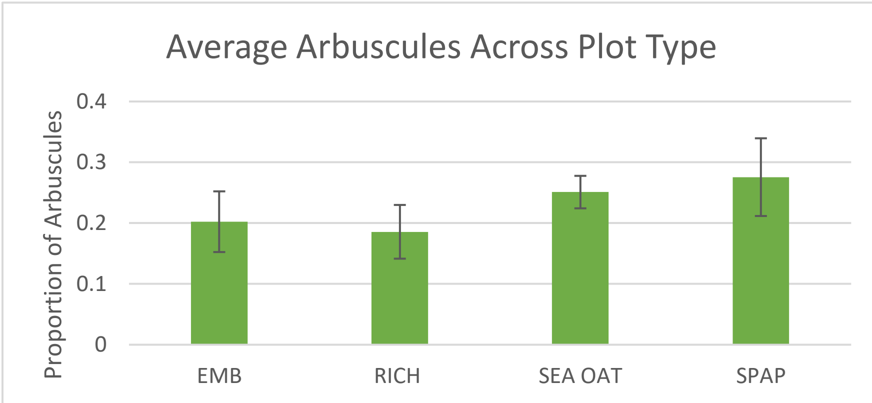

Figure 6. Illustration showing the average proportion of arbuscules across each plot type

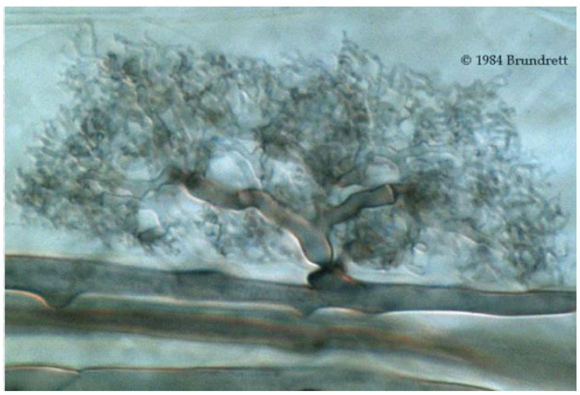

Figure 7. Image shows what arbuscules look like when seen under a microscope
(Marx, 1999).

Hyphae are a small tube like structures that grow from the spores of the fungi. The hyphae enter the host's roots through infection points and then forms extensive networks, which allow the mycorrhizae to stay attached to the root of the host plant (Marx, 1999). The hyphae absorb nutrients in the soil and transport them to the fungus body and then to the host (Malloch, 2019). Figure 8 shows an imagine of hyphae underneath a microscope.

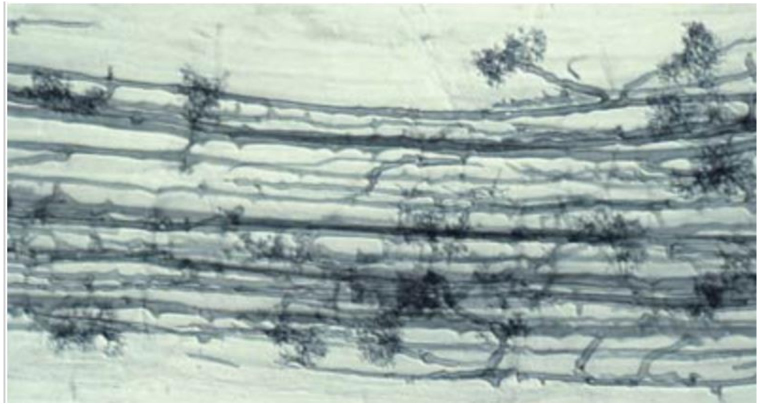

Figure 8. Microscopic image showing hyphae's tubular structures (Brundrett, 2008).

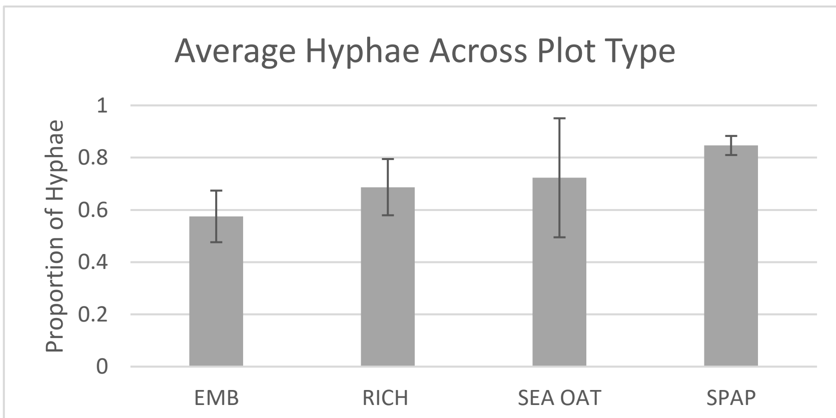

Figure 9. Illustration showing the average proportion of hyphae across each plot type

As seen above in Figure 9, roots from the "SPAP" plots had the highest levels of hyphae. About 85% of the roots seen in the plots labeled "SPAP" had the presence of hyphae in them. The embryonic dune patches had the least amount of hyphae, with about 56% of the roots being infected.

Discussion

Each plot showed some concentration of either hyphae, vesicles, or arbuscules, meaning that every plant was infected by mycorrhizae, not only plants higher on the bank as was hypothesized. Arbuscular mycorrhizae are routine inhabitants of dunes found in temperate and tropical climates which explains why every plot had high infection levels (Koske & Gemma, 1997). From the results shown above, it is evident that on average, the plots had the highest concentration of hyphae and the lowest concentration of arbuscules.

Findings from each site did not follow an exact trend and therefore it is difficult to state a clear reasoning for different levels of vesicles, arbuscles, and hyphae found throughout the plots. In the embiotic plots, there were very high levels of vesicles and the lowest concentration of hyphae. In the sea oat and SPAP plots, there were low concentration of vesicles and high concentration of hyphae. Between these three plots, there seems to be a slight trend: when there is high levels of vesicles, there are low levels of hyphae, and visa versa. This may be due to vesicles reinfecting dead root sections and starting new hyphal growth. The sea oat and SPAP plots showed similar levels for each mycorrhizal charactersitc: low levels of vesicles and high levels of arbuscules and hyphae. A reason why both plots have similar results may be due to both of the plots being located on the beachfront. The difference of infection rates across each plot type may be due to the fact that root colonization rates vary widely across different species and sampling location (Charbonneau, 2019).

Hyphae are the first to show up once infected and play a vital role in the host to fungi relationship, and thus have a long lifespan. With this is mind, the results found above make sense, since the hyphae had the highest concentrations throughout all the plots. Next, as stated before, arbuscules have a very short lifetime, and their branches deteriorate, explaining why throughout all the plots, there was very little concentration of arbuscules.

Conclusion

From the data presented above, it is evident the sand dunes on Sapelo Island are vastly colonized by mycorrhizae. Hyphae and vesicles were high across all root types, suggesting AM harvesting of resources is pervasive and there is an established history of association across all plant types. The roots also contained arbuscules but in a lesser amount than hyphae and vesicles. Because of this high level of infection, it is apparent this dune is in a relatively healthy, resilient state despite being exposed to two hurricanes. These plants are highly dependent on their symbionts to access water and nutrients so they can recover. Not only are the mycorrhizae fungi beneficial to the nutrient uptake of the plant, the fungi also assist in defending off harmful pathogens. Lastly, having high infection rates also makes the host plant less susceptible to environmental stresses including salinity. This is very beneficial to the plants on the dune because of their proximity to the ocean. These plots are on a sand dune that is constantly exposed to harmful anthropogenic effects, and have been exposed to two major hurricanes in the past five years, so it may be surprising that the roots were colonized so heavily by the fungi.

Acknowledgements

I would like to acknowledge Dr. Christine Angelini and the entire Angelini Lab Group for their constant support and help throughout this ongoing project. As well as Valerie Reijers for assisting with on-site sampling. Lastly, I would like to acknowledge the UF University Scholars Program for providing us this grant to complete our research.

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