GoNatives! Intern

Justin Mead
Western Washington University

COLLEGE OF THE ENVIRONMENT

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Student Name: Justin David Nicholas Mead

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STUDENT SIGNATURE ____________

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Investigating the Effects of Mycorrhizae on Sea Thrift Growth: Report on Internship with GoNatives! Plant Nursery

Justin D. N. Mead

Huxley College of the Environment, Western Washington University

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Dr. Rebecca Bunn

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Introduction

I was an intern for GoNatives!, a plant nursery that raises and sells local botanicals to local public and private entities. The owner of GoNatives!, Don Norman, is interested in the mycorrhizal associations of several local plant species, including *Rhododendrun macrophylum, Menziesia ferruginea, Cornus nuttallii, Paxistima myrsinites, Ceanothus velutinus, Achlys triphylla, Arbutus menziesii, Linnaea borealis,* and *Armeria maritima.* I conducted a literature search to determine what the scientific community knows about the mycorrhizal assoociations of each plant species. Based on the results of the literature search, I began an experiment with *A. maritima* (sea thrift), a perennial that thrives in coastal climates. The goal of my research was to determine if *A. maritima* is dependent on mycorrhizae and if soil inocula impact from different locations affect its growth. In this and similar studies, GoNatives! seeks to determine if their plants can benefit from the addition of mycorrhizal fungi.

Background Information

Mycorrhizal fungi associate with the roots of most plant species. They are successful mutualists in most environments. Mycorrhizal fungi benefit plants by enhancing water and nutrient uptake, especially in soils where these are sparser. They do this growing into plant roots and also extending into the soil. This increases both the surface area available for nutrient uptake and the volume of soil explored by plant roots. In return for greater access to nutrients, plants provide mycorrhizal fungi with carbohydrates. This relationship can be either obligate or facultative. Obligate mutualisms involve species that always coexist in their natural habitat, whereas facultative mutualisms form in certain ecological contexts but not others.

Mycorrhizae are classified by the morphology of the root-hyphae structures formed in the association between the fungi and host plant (Huey et al. 2020). The three kinds of mycorrhizae

GoNatives!'s plant species of interest form with mycorrhizal fungi are ectomycorrhizae (EcM), arbuscular mycorrhizae (AM), and ericoid mycorrhizae (ErM). Among the EcM, a subtype (Huey et al. 2020) known as arbutoid mycorrhizae are particularly common. AM and ErM are prominent varieties of endomycorrrhizae. EcM hyphae attach to the surface of root cells, anchoring themselves to their cell walls and associated structures (Huey et al. 2020). In contrast, endomycorrhizae hyphae penetrate both the cell wall and cell membrane of superficial root cells (Huey et al. 2020). AM fungi form characteristic structures called arbuscules within root cells which are the site of exchange between the fungus and the host plant. ErM are only known to associate with the roots of ericoid plants (Vohnik 2020). They are characterized by a bundle of coiled hyphae formed within the rhizodermal cells of root hairs (Vohnik 2020).

Due to their capacity to enhance plant growth, research and application of mycorrhizae may be beneficial to commercial and sustainability-related endeavors. Huey et al. (2020) discuss the potential role of mycorrhizae in sustainable agriculture. Applying fertilizer is one of the most common methods for maximizing crop yields, but it has the potential to substantially harm the natural environment and may be detrimental to agricultural productivity in the long run (Huey et al, 2020). By assisting crops with nutrient uptake, mycorrhizae may be able to reduce the need to apply fertilizer, thereby mitigating harmful impacts like eutrophication and toxic metal buildup in crops (Huey et al. 2020). Similarly, mycorrhizae may aid companies that sell native or exotic plants to public and private entities. This is the purpose of my research, as my employer seeks to understand how best to apply mycorrhizal fungi to grow native plants for commercial purposes.

Duties and Responsibilities

In fall quarter of 2021 and winter quarter of 2022, I conducted a literature search on the symbiotic relationships between mycorrhizal fungi and a list of local plant species from

GoNatives!. My primary goal was to find gaps in the literature to form a basis for future research. Existing studies show that *A. maritima* forms symbioses with AM fungi and that this relationship seems to have a positive impact on plant growth. Camprubi et al. (2011) found that *A. maritima* plants inoculated with AM fungi experience increased growth. Pawlowska et al. (1996) confirmed that *A. maritima* associate with AM fungi in their natural habitat, with between 17% and 30% root colonization.

We chose *A. maritma* because literature on mycorrhizal associations with this plant, although limited, suggest that it may benefit from mycorrhizae. In turn, GoNatives! and similar organizations can benefit from more direct research into whether mycorrhizal inoculation enhances the growth of this plant and what kinds of mycorrhizal fungi are optimal. Existing research into mycorrhizal associations with *A. maritima* may not be applicable to the conditions in which they would be grown in plant nurseries. Experimental studies often find that plants which are thought to form obligate mutualisms with mycorrhizae in the wild do not benefit from mycorrhizal inoculation in the laboratory. This likely arises from the fact that lab-based experiments involve more confined spaces than would be found in nature. Mycorrhizal hyphae enhance nutrient and water uptake by extending into the soil and increasing the surface area available for absorption. This is more beneficial when water and nutrients are diffuse, but these tend to be concentrated in laboratory experiments. This illustrates the importance of environmental conditions in determining the relative impact of mycorrhizae on plant growth. As a result, it is critical that experimental conditions are similar to those in which the plants would be cultivated in plant nurseries.

In March, I began the experimental phase of my internship. I planted 50 *A. maritima* seeds in vermiculite in Western Washington University's greenhouse and 50 in petri dishes. The petri dish seeds were lightly encapsulated by a sheet of filter paper on the top and bottom. I watered both the greenhouse and petri dish seedlings daily. Additionally, I monitored and recorded any signs of stress, including nutrient deficiency, pest infestation, and osmotic stress. Other duties included ordering some of the supplies, such as seed trays, and cleaning equipment. My first attempt at sprouting *A. maritima* was unsuccessful, but my second was. Another student used the seedlings from my second attempt to complete the experiment I intended to carry out (see below).

After allowing the seeds to sprout, I would apply treatments and commence the experiment. The treatment groups were to include a sample of seedlings grown with commercial inoculum and another grown with whole soil inoculum collected on-site. Adding to this, I would have a control group of seedlings grown in sterilized growth medium without any microbial inoculum. To collect the whole soil inoculum, I identified nearby sightings of *A. maritima* reported on iNaturalist. After three months of growth, I was to harvest the *A. maritima* plants and measure their dry mass. Once I collected all the data, I would compare the average dry mass amongst the treatment groups' root and shoot systems. I would also measure plant height as a response variable.

Outcomes

Literature Review

Of the native plant species on GoNatives!' list, all but two (*R. macrophyllum* and *A. menziesii*) were found to associate with AM fungi (Table 1). Additionally, the literature finds or assumes that all of these species form obligate mutualisms with mycorrhizal fungi, except for *C. nuttallii* and *A. maritima* (Table 1). The latter two species have a facultative relationship with mycorrhizal fungi (Table 1). This was among the main reasons for choosing *A. maritima* as the model organism for this study. Plants that form obligate mutualisms with mycorrhizal fungi do not grow in their absence, at least in their natural habitat. In turn, a study on the impact of mycorrhizal inocula on the growth of plants in obligate mutualism with these fungi would provide little useful information. Although *C. nuttallii* is also a facultative mutualist of mycorrhizal fungi, it is difficult to experiment with due to its large size.

Table 1: Summary of literature search information, including relevant basic information about each species on GoNatives!' interest list and the type of evidence for their mycorrhizal associations. "EcM" refers to ectomycorrhizae, "AM" to arbuscular mycorrhizae, and ErM to ericoid mycorrhizae.

Species	Common Name	Functional Group	Habitat	Mycorrizhal Type	Mycorrhizal Dependency (Family-Specific; Wang & Qui 2006)	Taxonomic Level of Classification for Mycorrhizal Type	Evidence Type (Molecular or Microscopic)	Study Type (Lab or Field)
Arbutus menziesii	Madrone	Tree	Forest	EcM - Arbutoid (Massicotte et al. 1993, Molina & Trappe 1982, Zak,1976)	Obligate	Species	Microscopic	Field
Menziesia ferruginea	False Azalea	Shrub	Forest	EcM (Stoyke & Currah 1993)	Obligate	Species	Microscopic	Lab
Cornus nuttallii	Pacific Dogwood	Tree	Forest	AM (Wang & Qui 2006)	Facultative	Family	Molecular	Lab

Paxistima myrsinites	Oregon Boxwood	Shrub	Forest	AM (Gorzelak et al. 2017)	Obligate	Species	Molecular	Field
Rhododendron macrophyllum	Rhododendron	Shrub	Forest	ErM (Molina & Trappe 1982)	Obligate	Genus	Microscopic	Lab
Ceanothus velutinus	Buckbrush	Nitrogen-fixing shrub	Forest and grassland	AM (Rojas et al. 2002, Rojas-Melo 1997, Rose & Youngberg 1981)	Obligate	Species	Microscopic	Lab
Armeria maritima	Sea Thrift	Perennial	Grasslands and coastal	AM (Palowska et al. 1996, Camprubi et al. 2011, Hildebrandt et al. 2001)	Facultative	Species	Microscopic	Field
Linnaea borealis	Twinflower	Perennial	Forest	AM (Kranabetter & Mackenzie 2010, Gorzelak et al. 2017)	Obligate	Species	Microscopic	Field

Achlys triphylla	Vanilla Leaf	Perennial	Forests and along streambeds	EcM (Elliott et al. 2007)	Obligate	Genus	Microscopic	Field

A. maritima belongs to the family Plumbaginaceae, which was not initially thought to form mycorrhizae (Hildebrandt et al., 2001). However, Camprubi et al. (2011) and Pawlowska et al. (1996) found *A. maritima* were colonized by AM in the field, especially by *Glomus intraradices*. Hildebrandt et al. (2001) found no arbuscules in the roots of collected *A. maritima*, but suspect that this may have arisen from the fact that they collected their samples at the end of the year when photosynthetic rates are slowing down.

Other noteworthy findings from the literature search involve plant associations with Actinomycetota and dark septate endophytes. Specifically, Actinomycetota may promote *C.velutinus* growth when combined with AM inoculation (Rose and Youngberg 1981), though one study found no evidence of this association (Rojas et al. 2002). The reason for the discrepancy is unclear. Rojas et al. (2002) offer no suggestions as to why their results differ from those of Rose and Youngberg (1981), though their comments on slope effects and the differences between *Alnus rubra* and *C. velutinus* responses may provide some insight. Namely, Rojas et al. (2002) speculate that the two plant species may respond preferentially to different strains of *Frankia*, which may be distributed unevenly across the sample collection site. Additionally, they note that *C. velutinus* were sparsely distributed at the site, and that this is likely the result of some characteristic in the soil brought about by a recent clear-cut and slash-burn in the area. In turn, their results may differ from those of Rose and Youngberg (1981) because of a factor in the soil that was limiting *C. velutinus* growth despite inoculation with AM and Actinomycetota. This also raises the issue of experimental control. The fact that Rojas et al. (2002) incorporated site-collected soil as opposed to a sterile growth medium introduces the potential for some systemic error and increased random variation in their results. Further research is needed to assess associations amongst *C. velutinus*, mycorrhizal fungi, and nitrogen-fixing bacteria.

Dark septate endophytes are a type of fungi commonly observed colonizing plant roots in alpine, subalpine, and artic habitats, or other environments with cold soil (Stoyke and Currah 1993). These fungi are not yet well-understood, but they seem to have a positive impact on plant growth in certain circumstances (Stoyke and Currah 1993, Jumpponen et al. 1998). Stoyke and Currah (1993) found that *Genococcum geophilum*, a dark septate endophyte, formed ectomycorrhizal associations with *M. ferruginea* samples collected from subalpine soils. However, this was the only dark septate endophyte which exhibited mycorrhizal growth patterns in their study. Interestingly, *Phialocephala fortinii*, another dark septate endophyte, has demonstrated pathogenic tendencies in some contexts (Stoyke and Currah 1993). Despite there being no clear evidence of mutualism, this fungus is prevalent in subalpine communities, suggesting that it is ecologically significant (Stoyke and Currah 1993). Further research is needed to understand the nature of symbiotic relationships between dark septate endophytes and cold-soil plant species.

Sprouting Trials

The *A. maritima* seedlings underwent little growth. This was partially because *A. maritima* is a wild plant. Wild plants have not undergone the artificial selection and genetic modification that allow domesticated plants to grow more rapidly than they would in nature. Other factors that may have limited the seedlings’ growth include the depth at which I planted them, inconsistent watering regimes, the growth medium, and ambient conditions in the greenhouse. As a result, I was unable to attain substantive data. However, my experience provided me with valuable information about how to encourage *A. maritima* seedlings to sprout efficiently.

One of my key takeaways from sprouting *A. maritima* is that the seeds must not be buried deeply in the growth medium. I had the greatest success sprouting seeds that were covered by only a thin layer of medium. In the future, it may be wise to attempt planting the *A. maritima* seeds without a layer of growth medium covering them and compare sprouting success to that of thinly covered seeds. I also found that petri dishes were unsuitable for sprouting *A. maritima*, because they readily became overrun with anaerobic microbes when I kept the lid on and rapidly desiccated when I left the lid off. Additionally, the *A. maritima* sprouted more readily in a growth medium consisting of 70% fine bark and 30% peat moss by volume than in pure vermiculite. Finally, due to variable weather, I was unable to establish a watering regime that consistently met the seedlings' needs. It was apparent, however, that I watered them excessively toward the beginning of the experiment. Incorporating the advice of my internship advisor and my own observations and judgement, I determined that it is better to under-water the plants than to over-water them. The adverse impact of under-watering the seedlings may be minimized by checking them daily for dehydration, but over-watering can kill the plants via suffocation. Dry cells can be watered, but excess water cannot be physically removed.

Assessment

I was unable to sprout enough seedlings to begin the experiment as intended. if I was to reattempt the experiment. Importantly, I found that the seedlings sprouted more quickly and consistently when they were covered by only a thin layer of growth medium. They also sprouted better when I used a growth medium consisting of 70% fine bark and 30% peat by volume instead of vermiculite. It is difficult to differentiate between the effects of the type of growth medium and depth of seed planting, as I most likely planted the seeds more shallowly when using the second growth medium. A future experiment could test growth medium and seed depth separately to determine the independent effects of these variables on sprouting success.

I found that seeds sprouting in petri dishes are more vulnerable to osmotic stress and infestation by unwanted microorganisms. Toward the beginning of the experiment, I attempted to sprout these seeds with the lid on. They soon developed signs of colonization by mold and anaerobic bacteria. In response, I began leaving the lid ajar after watering the seeds to allow for airflow. As a result, they became more prone to desiccation. Within one day, the trays could go from being moist to completely dehydrated. After several weeks of accumulated osmotic stress and microbial infestation, it became clear that the seeds were unsalvageable, and I disposed of them.

Learning from my mistakes, I am confident that I would be more successful if I repeated the experiment. In a second attempt, I would once again plant *A. maritima* seeds in a 72-cell tray. I would still plant two seedlings per cell, grow the seedlings in the greenhouse, and water them once or twice daily as needed. By keeping these factors the same, I would be able to develop a better understanding of how to optimize *A. maritima* growth for future trials. In contrast with my first attempt, I would plant 200 seeds in 72-cell trays and none in petri dishes, use the 70% fine bark and 30% peat growth medium, and cover the seeds with only a thin layer of growth medium. I would also allow for more time to complete the experiment. Initially, I wanted to complete the experiment in about one quarter, failing to recognize how slowly wild *A. maritima* seeds sprout and grow. My modified timeline would include 10 weeks of seed sprouting and 15 weeks of growth following application of treatment, with up to 10 weeks of data collection and analysis. This timeline allows for a margin of error, as a school year is almost 40 weeks long and data collection and analysis would not take 10 weeks to complete.

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