EFFECTS OF NUTRIENT LEVELS ON THE COLONIZATION OF *POA SECUNDA* BY ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTES

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Abstract

Arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) are both root-inhabiting fungi that form symbiotic relationships with host plants. Depending on environmental conditions such as soil nutrient levels, the symbiosis can range from mutualistic to parasitic. To examine effects of nutrient levels on the colonization of AMF and DSE, *Poa secunda*, a native grass species, was grown in potting media inoculated with soil from the root zone of *P. secunda* individuals, expected to contain both types of root fungi. Soil nutrient level was then manipulated using commercial slow-release fertilizer (Osmocote) to three concentrations: 100% of the recommended rate in high nutrient cones, 50% of the recommended rate in medium nutrient cones, or none with no added nutrients. Host roots were then examined to assess percent colonization by AMF and DSE at each nutrient level. The results showed AMF colonization increased with increasing nutrients, while DSE colonization did not correlate significantly with nutrient levels.

*Keywords:* Ecology, Fungi, Nutrient Variation, Arbuscular Mycorrhizal Fungi, Mycorrhizae, Dark Septate Endophytes

INTRODUCTION

**MYCORRHIZAE** are symbioses between fungi and the roots of vascular plants. In a survey done by B. Wang of 659 published papers, it has been estimated that of the 3,617 species of plants mentioned, approximately 92% were hosts to mycorrhizal fungi; making mycorrhizae the most prevalent symbiosis within the plant kingdom [1].

This symbiosis works as mycorrhizal fungi colonize plant’s roots, which can be done intracellularly known as arbuscular mycorrhizal fungi or extracellularly as in ectomycorrhizal fungi. This plant-fungus symbiosis can range along a continuum, ranging from mutualistic to commensal to parasitic depending on developmental factors, environmental conditions, and genetics. Because mycorrhizal fungi are obligates, meaning they rely on the host plant in order to survive, they will always have a positive response in this association. The host plant on the other hand will have a positive response (mutualistic), neutral response (commensal), or negative response (parasitic) to this symbiosis.

The majority of mycorrhizal-plant interactions are mutualistic, meaning both the plant and fungus benefit from the interaction. Plants benefit from increased access to soil nutrients, increased drought tolerance, and enhanced pathogen resistance. When mycorrhizal fungi colonize a host plant’s roots they form very thin filaments that increases the root’s surface area allowing mycorrhizal hyphae to access nutrients and water that may be inaccessible to roots alone. This can be
beneficial to the plant because it allows the roots to be more efficient at getting nutrients like phosphorus and nitrogen and especially immobile nutrients like copper, zinc, iron, and manganese [2]. This increased efficiency is especially important in areas with low nutrient soils, because mycorrhizal hyphae are capable of going meters deep in order to absorb these nutrients. Host plants also receive protection from root infecting pathogens. This can be done by the fungal hyphae by secreting chemical antibiotics or by occupying root space that may have been occupied by a pathogen or nematode had the mycorrhizal hyphae not been there [3]. Another important benefit to the plant is the connection of mycorrhizal hyphae between different plants, thus creating a mycorrhizal network that allows the transport of nutrients between multiple plants. For example one seedling can tap into the mycorrhizal root system of a parent tree and use their nutrients as its getting established, which is an important interaction used in ecological restoration. In return, mycorrhizal fungi receive carbohydrates in the form of sugars, which are formed via photosynthesis by the host plant.

Parasitism occurs when the host plant is negatively impacted while the mycorrhizal fungi is benefiting. There are three main hypotheses for why this normally mutual symbiosis becomes parasitic. The first form of parasitism may result from developmental factors that arise from the temporal relationship of the plant and mycorrhizal fungi. Next are environmental factors, represented by the conditions outside the plant-mycorrhizal complex. Lastly parasitism could be due to genotypic factors of the host plant or its colonizing mycorrhizal fungus [3].

The fungus can become parasitic to the host plant at certain stages in development of the mycorrhiza. For example, while the mycorrhizal hyphae are forming, the plant will be allocating some of its photosynthesize for the developing mycorrhizae, but the plant is still unable to reap its benefits from the mycorrhizae [3]. At this time the seedling could be decreasing its own growth because of the low resources resulting from the allocation of carbon. These short-term losses are soon compensated with long-term gains, once mycorrhizal hyphae are formed. The amount of carbon that is being produced through photosynthesis by the plant during this stage is also dependent on environmental conditions.

The mycorrhizae can also become parasitic depending on the amount of resources available for translocation by the hyphae, to the plant. The most studied environmental factor is the nutrient status of the soil. A parasitic interaction can arise when there are little nutrients available in the soil, but also when soil nutrients are very high. First when soil resources are limited, the fungus will continue to take the same amount of carbon from the plant, but since there are fewer resources in the soil the fungus is not providing as many benefits back to their host plants. This interaction becomes parasitic when the cost of producing carbon that the fungus receives exceeds the nutrients that the fungus supplies to the plant [3]. High soil nutrient levels can also cause a parasitic interaction. This can be seen with human’s excessive use of fertilizers that are nutrient rich. This results in mycorrhizal fungi becoming superfluous and unnecessary because plant roots alone would be able to uptake the nutrients needed by the plant [3]. This interaction then becomes parasitic because the fungus continues to take carbon from the plant, but the plant no longer needs the fungus for its resource acquisition. Thus once again the cost of maintaining the fungus is greater than the benefit they provide.

There are also genetic factors that can lead to parasitism. Although 92% of all plants are mycorrhizal, the degree of benefits received from the mycorrhizal interaction depends in
part on the plants genotype [1]. For example plant genotypes with coarse roots benefit more from mycorrhizal colonization than genotypes that have fibrous root systems. This leaves 8% of plants having no benefits or no preference for having a fungal associate, however this is rare in nature and the majority of plants are mycorrhizal to some degree [4]. These truly non-mycorrhizal plants have negative responses such as decreased growth and root necrosis when inoculated with mycorrhizal fungi.

There are over 600 species of plants known to be hosts of DSE [5]. The function of DSE has not been thoroughly understood; it has only been concluded that endophytic fungi don't cause any negative effects to their host plants [5].

In a study done by Jumpponen, P. fortinii was grown in soil inoculated with DSE and given nitrogen fertilization resulted in an increased plant biomass than when treated with nitrogen fertilization alone [6]. This study is one of the few to show the effects DSE colonization has on host plants.

**OBJECTIVE**

The purpose of this study is to determine the percent colonization by arbuscular mycorrhizal fungi and dark septate endophytes at varying nutrient levels.

**HYPOTHESIS**

I predicted that AMF colonization would be higher in low nutrient levels because prior research has shown that at low nutrient levels the relationship between mycorrhizal fungi and their host plant is mutualistic. Conditions influencing endophytic symbiosis have not been studied extensively due to endophytes' intracellular growth, making it difficult to visually observe endophytes' effects on plant growth. However, I predicted that DSE colonization to be higher in high nutrient levels, as there will be less competition with AMF.

**METHODS**

*Poa secunda*, a native grass species, was grown in Profile potting media inoculated with soil from the root zone of *P. secunda* individuals. The mixture was 1/10 live soil and 9/10 Profile. Soil was collected from Quail Creek Reserve on Lake Berryessa in Northern California coast range, and was expected to contain both types of root fungi. Soil nutrient levels were then manipulated using commercial slow-release fertilizer, Osmocote, to three concentrations, each a percentage of the recommended rate. High nutrient pots were 100% of the recommended dose at 0.39g, medium nutrients were 50% of the recommended dose at 0.19g, or none at 0g added nutrients. A total of 36 samples were planted with 12 cones in high nutrients, 12 cones in medium nutrients, and 12 cones with no added nutrients.

Six *P. secunda* seeds were grown in each of the SC10 super Stuewe & Sons 164mL cones. They were allowed to germinate and grow in a greenhouse for 10 weeks, where they were watered daily with de-ionized water and kept at temperatures between 18 and 32 Celsius. In total, 16 of the 36 samples germinated. The germinated plants were thinned to one plant per pot. After 11 weeks the roots and shoots were carefully harvested and washed.

Roots were cut from the shoots and stored in histology cassettes in de-ionized water till they underwent a modified version of Vierhielig et al. hot dye staining method [7]. To begin the dying process cassettes were soaked in nearly boiling 10% KOH for five minutes to clear the cells of organelles. The cassettes were then drained and rinsed with de-ionized water. Then they were soaked for 12 hours in household vinegar to acidify roots. Next, the cassettes were placed in a near-
boiling solution of Parker Quink Blue-Black ink/vinegar for three minutes to stain the roots. Finally, roots were very briefly washed with 0.5% KOH to remove the extra dye. Once dyed, the roots were mounted on slides and viewed under a compound light microscope at 400x magnification following McGonigle et al. [8]. The number AMF and DSE were counted at 100 points in each slide to determine the percent colonization. At each of the 100 counts the crosshatch is tallied for the presence of AMF, DSE, or nothing.

RESULTS

Of the 16 germinated samples six were from high nutrients, five from medium nutrients, and five from no added nutrients. The results from Figure 1 illustrate a slight increase in AMF colonization with increasing nutrients. However no correlation was visible between DSE colonization and nutrient levels.

DISCUSSION

Data show that DSE colonization remained consistent through the three nutrient levels, while mycorrhizal colonization increased with increasing nutrients. This is surprising since past research has determined that mycorrhizae are the most beneficial at low nutrient levels. These unexpected results could be because the P. secunda seeds were only grown for 10 weeks, not giving AMF enough time to become beneficial to host plants. This may indicate developmental parasitism and might occur because P. secunda plants were providing root fungi with sugars, while the root fungi were not benefiting the plants as they were still colonizing. At low nutrient levels the mycorrhizal fungi was receiving carbon from the host plant, but the plant was receiving little nutrients in return, thus were not able to support as high of a colonization rate as plants grown in high nutrient levels. At high nutrients, host plants are giving carbon to the mycorrhizal fungi, but because there are more
nutrients available, the host plant is able to support more colonization. The experiment's methods may have also affected the results. The sample size was small, with only 12 samples at each nutrient level for a total of 36 samples. Had there been a larger sample size, the data may have resulted in a stronger correlation.

Ultimately, the data from this experiment is inconclusive, although it may be correct that there is not a strong relationship between fungal colonization and nutrient levels, research on mycorrhial fungi has shown otherwise. Had there been a negative correlation or increased colonization at low nutrients, mycorrhizal fungi could be used in restoration. If colonization increased and persisted at low nutrients it would show a mutualistic relationship between AMF and host plants. Meaning that at low nutrients, AMF could provide host plants with increased access to nutrients to promote the growth of host plants. This would result in more productive biota in low nutrient locations that may not have been suitable for plant growth.

Interpretation of the lack of variation in DSE colonization in response to the different nutrient levels is difficult, as DSE have not been extensively studied. Further research on DSE and the alkaloids they secrete could show their biological potential, possibly leading to natural products for the use in medicine, industry, and agriculture.

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REFERENCES