RESPONDING TO SOIL FUNGAL COMMUNITIES: A LOOK AT INTERACTIONS BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND THE COMMON YELLOW MONKEYFLOWER

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RESPONDING TO SOIL FUNGAL COMMUNITIES: A LOOK AT INTERACTIONS BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND THE COMMON YELLOW MONKEYFLOWER

By

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Undergraduate thesis presented in partial fulfillment of the requirements for the University Scholar distinction

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Missoula, MT

Approved By:
Dr. Lila Fishman, Department of Biological Sciences
Abstract
McIntosh, Mariah, B.S., May 2017
Faculty Mentor: Lila Fishman

The obligate fungal mutualists arbuscular mycorrhizal fungi (AMF) colonize the roots approximately 80% of vascular plants, generally thought to provide mineral nutrition, pathogen protection, or drought resistance to plants in exchange for photosynthetic carbon. Because of the ecological and evolutionary significance of these interactions, much work has been done to understand this symbiosis at the community level. However, much remains to be understood about how AMF affect plant fitness on an individual level. In this study, we took advantage of the tractability of the emerging model species Mimulus guttatus, the common yellow monkeyflower, to identify genetic differences in how contrasting annual and perennial populations respond to AMF. Specifically, we tested for differences in plant dependency on AMF, variation in local adaptation to native AMF communities, and ability to selectively associate with some AMF taxa over others. We conducted a full factorial common garden greenhouse experiment using plant, soil, and inoculum from each contrasting field site. We found no dependency on AMF in either population and no local adaptation to native AMF communities; however, we did find some subtle differences between contrasting annual and perennial plant types in how they interact with AMF. The presence of AMF did not confer a fitness advantage to either plant type and was often associated with a fitness cost, despite differences in life history, providing evidence for a potentially antagonistic relationship between M. guttatus and AMF under certain conditions, consistent with the theory that more ruderal species are less likely to benefit from AMF.
Introduction

Plant-AMF Interactions

Symbionts are a fundamental part of life on Earth. From the vertebrate microbiome to the underground nests of leafcutter ants, symbiosis takes many forms. Arbuscular mycorrhizal fungi (AMF), a group of highly abundant fungal symbionts, colonize the roots of 80 percent of vascular plant species (Smith and Read 1996). AMF are integral to plant nutrient uptake, pathogen protection (Newsham et al. 1995), and stress tolerance. AMF greatly influence plant diversity (Grime et al. 1987), community composition (Klironomos et al. 2011), ecosystem variability, and overall productivity (Smith & Read, 1996; van der Heijden et al., 1998). Therefore, this important mutualism strongly affects plant ecology, with implications for agriculture (Ryan and Graham 2002), ecological restoration (Klironomos, 2003) and plant responses to climate change (Compant, et al., 2010).

Previous work has shown large variation in plant response to AMF, with some plants greatly benefiting from this association, other exhibiting a cost, and yet others showing no cost or benefit when grown with AMF (Klironomos, 2003; Eo & Eom, 2009). Plant ecologists have used broad plant functional traits to try to explain this variation, including root characteristics (Bardgett et al. 2014), leaf mass per unit area (Waller, 2015), and plant life history (Wilson & Harnett, 1998). However, these studies largely characterize this variation at a macroevolutionary, community level, looking for variation among highly differentiated taxa. Currently we do not understand how these patterns evolve at a microevolutionary, individual level, and if there are differences between very closely related taxa. This study uses two contrasting annual and perennial populations of the highly diverse and well-characterized plant Mimulus guttatus, the common yellow monkeyflower, to identify genetic differences in how plants perform with and without AMF (dependency), to what degree plants and their local fungal communities have coevolved (local adaptation), and if plants are able to select their fungal partners preferentially (specificity). These mechanistic components of plant-AMF interactions strongly affect plant fitness, meaning that they are critical for both a basic understanding the plant-AMF systems, and for effective use in agriculture, ecological restoration, and more (Figure 1).

Figure 1. Conceptual diagram showing mechanisms of plant-AMF interactions.
Study System

*Mimulus guttatus*, the common yellow monkeyflower, is an ideal system to study these questions because of its diversity, genomic resources, and tractability (Wu et al. 2008). We focused on two well-characterized, contrasting ecotypes from Oregon, already studied by Dr. Lila Fishman: an alpine annual, and a low elevation perennial. The Iron Mountain (IM; Figure 2) high elevation (1463 m; Willis, 1993) annual population in the Oregon Cascades is characterized by small, fast-growing plants dependent on snowmelt to avoid drying under the hot summer sun (Hall and Willis 2006), which commonly produce no more than one flower and fruit each year (Fishman and Willis 2008). Plants flower in mid- to late-July, when temperatures reach above 40˚C (Hall and Willis 2006). Winter temperatures at IM reach below freezing, and the site accumulates on average 5m of snow per year (Hall and Willis 2006).

The contrasting low elevation (sea level) perennial population is found along the Oregon Coastal Dunes (DUN; Figure 3), where larger, long-lived plants experience a more moderate climate, and exhibit a slower life history, spreading through vegetative branches, and overwintering as rosettes. DUN plants germinate in fall and flower from June until late fall. The climate at DUN is temperate year-round, with less than 20˚C fluctuation annually, and moisture from rain, fog, or shallow pools available throughout the year (Hall and Willis 2006).
### Soil Analysis

<table>
<thead>
<tr>
<th></th>
<th>IM Soil</th>
<th>DUN Soil</th>
<th>Healthy Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.4</td>
<td>6.3</td>
<td>6.0-6.5</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>7.6</td>
<td>0.4</td>
<td>4.0-10.0</td>
</tr>
<tr>
<td>Nitrogen (ppm)</td>
<td>2.6</td>
<td>0.1</td>
<td>20-40</td>
</tr>
<tr>
<td>Phosphorus (ppm)</td>
<td>9</td>
<td>7</td>
<td>20-40</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>182</td>
<td>76</td>
<td>150-250</td>
</tr>
<tr>
<td>Sulfur (ppm)</td>
<td>10</td>
<td>4</td>
<td>5-20</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>.38</td>
<td>.42</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>11.2</td>
<td>1.2</td>
<td>1-5</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>.68</td>
<td>.05</td>
<td>.6</td>
</tr>
<tr>
<td>Magnesium (ppm)</td>
<td>775</td>
<td>52</td>
<td>60-300</td>
</tr>
</tbody>
</table>

*Table 1.* Soil nutrient analysis performed by Wards Laboratories. Green signifies the value is within a healthy range, red signifies the value is low, yellow signifies the value is close to the healthy range, and blue signifies the value is high, as compared to nutrient values provided by the Oregon State University Extension Agency (2011).

While both sites are relatively nutrient deficient (Table 1), their soil characteristics differ significantly: IM soil is shallow, porous, and rocky (Hall and Willis 2006), with some organic matter, while DUN soil is little more than sand. The sites share approximately the same latitude. Preliminary data have shown that roots of M. guttatus from both sites are colonized by AMF in the field (Table 2, in Results), and spores are present in the soil. These ecotypes are known to be locally adapted to their respective environmental conditions (Hall and Willis 2006).

These contrasting, but closely-related ecotypes allow us to better understand the differences in how annual and perennial populations interact with AMF, and whether or not these differences are genetic. While much work has characterized differences between annuals and perennials, as well as other plant traits, on a macroevolutionary scale (Klironomos, 2003; Reinhart, Wilson, & Rinella, 2012), our experiment tests for changes in how plants interact with AMF on the microevolutionary scale.

#### Questions

1. **Dependency**—Is there a genetic difference in the benefit M. guttatus ecotypes receive from AMF?
2. **Local Adaptation**—Does M. guttatus perform better in its native mycorrhizal community versus an exotic mycorrhizal community?
3. **Specificity**—Do plants select and associate with only a subset of fungal partners from those available in soil communities?

#### Predictions

1. **Dependency**—Research shows that some taxa gain little fitness advantage from association with AMF (low dependency); others gain a lot (high dependency; Reinhart et al., 2012). Life history strategies may influence AMF dependency, with perennials generally more dependent on AMF than annuals.
(Reinhart et al., 2012; Figure 4A). Therefore, DUN plants will benefit more from mycorrhizae than IM plants.

2. **Local Adaptation**— AMF community composition varies across soil gradients (Birkhofer et al., 2012; van der Heijden et al., 1998), and plants respond variably to different AMF taxa (Klironomos, 2003), including native versus exotic AMF (Klironomos, 2003; Schechter & Bruns, 2013). Therefore, we suggest that AMF-plant interactions may contribute to increased performance in native soils (local adaptation, Figure 4B). Therefore, both IM and DUN plants will perform better in their native mycorrhizal communities than in exotic mycorrhizal communities.

3. **Specificity**— Some AMF may be better partners than others by providing more mineral nutrients or other benefits per unit of photosynthate (Kiers & Heijden, 2006; Smith, Smith, & Jakobsen, 2003), and plants may preferentially associate with certain AMF species from a given community (specificity; Figures 4C & 5). Therefore, AMF species found in the roots IM and DUN plants will be a subset of those found in the soil.

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**Figure 4.** Predicted results. **A)** If perennials are more dependent on AMF, DUN (but not IM) will perform relatively better grown in soils with versus without AMF. **B)** If populations are locally adapted to AMF communities, each will perform better when grown with AMF from their native soil. **C)** If populations exhibit specificity, AMF communities (characterized by amplicon sequencing) isolated from within roots of IM and/or DUN will be a subset of those species available in the soil.
Methods

Full Factorial, Common Garden Greenhouse Experiment
To test for dependency and local adaptation, we conducted a full-factorial greenhouse common garden experiment (Figure 6) varying soil type (DUN or IM sterile background soil), plant type (DUN/IM ecotype), and inoculum (DUN, IM, DUN&IM, a general mycorrhizal inoculum, and no inoculum), with n=12 of each treatment. We collected bulk field soil to use as fungal inoculum in July of 2015 from both DUN and IM field sites. After transportation at room temperature in plastic bins, the soil remained under refrigeration until use. To generate more concentrated mycorrhizal inoculum, we grew *M. guttatus* from each field site in 4” pots in the greenhouse from seed in its respective inoculum mixed with Turface and sterile sand (1:1:1).

For the general mycorrhizal inoculum (GEN), we used inoculum from Ylva Lekberg generated by growing *Panicum vulgare* in 1:1:1 mix of autoclaved field MPG Ranch (Missoula, MT) field soil, sand, and Turface for three months inoculated with spores to amplify individual species of AMF, and then mixing all individual species to make a mixed grassland community. To create nonmycorrhizal control inoculum, we inoculated identical pots filled with sterile field soil, Turface, and sterile sand (1:1:1) with 20mL soil slurry containing bacteria and other microbes, but no AMF, derived from 50mL of live field inoculum mixed with water and filtered twice through a 250-micron sieve and filter paper. We allowed these trap cultures to grow for approximately three months in the greenhouse under short days to promote maximum fungal growth. We fertilized each pot with 20mL of 20-2-20 fertilizer at 50ppm N every two weeks and watered them every-other day. After allowing the plants to die and the pots to dry out, we bagged the dry inoculum in Ziploc bags and stored it at room temperature until use.
We germinated plants destined for my full factorial experiment in petri dishes filled with sterile sand and water in the fridge. After one week in the fridge, we moved them to a south-facing windowsill, and after a week more, they were housed in the greenhouse under long days until planting. To prepare pots for my experiment, we lined 200mL Cone-Tainers with paper towels to prevent leakage, added 100mL of sterile field soil corresponding to inoculum type, Turface, and sterile sand (1:1:1), 30mL of live inoculum generated previously, and topped off the pot with the same sterile soil, Turface, and sand mix.

We transplanted one four- to five-week-old *M. guttatus* seedling of the correct site origin carefully with forceps into each pot. We replanted seedlings that died up to three times within ten days of the original transplanting. In the greenhouse, we randomized my experiment by treatment to avoid greenhouse effects, but with respect to inoculum type to avoid contamination. We allowed the plants to grow under long days with daily or twice-daily top watering (depending on greenhouse temperature) and fertilization with 10mL of 20-2-20 fertilizer at 50ppm N every two weeks and then every week beginning mid-June.

Prior to harvest, the date of the first flower and the total number of flowers and buds (added to determine number of potential fruit) were collected. On July 16th, after
approximately eleven weeks, and once all plants had the opportunity to flower, we began harvest. We removed plants from their pots, removed soil from their roots, separated below- and above-ground biomass, subsampled roots for molecular analysis into 96-well CoStar plates on ice, and prepared biomass for drying. We dried below- and above-ground biomass samples in envelopes in a drying oven at 70˚C until dry. We weighed all dried samples. We performed an ANOVA (analysis of variance) in the statistical program JMP to analyze the results of this experiment, including soil type, plant type, and either inoculum origin and mycorrhizal or nonmycorrhizal, or microbes, which sorted each inoculum, AMF positive or negative, separately (i.e. DUN M, or DUN NM).

Molecular Analysis of Field and Greenhouse Samples
To test for specificity, we conducted a molecular analysis of field roots and soil, the inoculum we used in my experiment, and roots from the greenhouse experiment (Figure 5) using next-generation sequencing. Paired soil and root samples (n=20 per site) were collected in the field at IM and DUN sites in June of 2016. Roots from 20 subsites at each site were collected on ice and separated into samples for molecular analysis, which were later rinsed and frozen at -80˚C in 2mL tubes, and samples for root staining, which were dried in an oven for approximately 40 minutes at 75˚C and stored in envelopes at room temperature until use. Soil samples from the same subsites were collected into 15mL tubes on ice and frozen at -80˚C until use. After collecting root samples from my greenhouse experiment on ice, we stored them at -80˚C until use.

We extracted DNA from all root samples using a CTAB-chloroform protocol adapted for 96-well plates. For field roots, we freeze dried them prior to extraction, rather than using liquid nitrogen, as was used for greenhouse root samples. We extracted DNA from all soil samples, including the inoculum used in my greenhouse experiment using Mo Bio PowerLyzer PowerSoil DNA Isolation Kits. Because the small soil volume recommended by the kit (200 mg) did not amplify, we filtered organic matter, including hyphae and spores present in the soil, from 15mL of soil by mixing it with deionized water, filtering large particles including soil, rocks, and root pieces out using a 250 micron asieve, discarding the what did no precipitate. This precipitate (the whole sample, up to the recommended 250 mg) was added to the PowerLyzer PowerSoil kit, and we proceeded as usual with the extraction following the manufacturer’s instructions.

Each samples were amplified using a nested PCR I protocol, run with both AML2/WANDA (AMF-specific) and ITS4/ITS7 (fungal-specific) primer pairs. We checked amplification of all samples in PCR I using gel electrophoresis. Then, PCR product was diluted based on band strength and adapters were ligated to each sample in PCR II. All samples were pooled by DNA concentration and sent to the iBEST Genomics Core Facility at the University of Idaho for sequencing. After sequencing, samples will be de-convoluted as assigned to taxonomic units based on amplicon sequence, and we will analyze soil and root community characteristics.

Colonization
Roots were checked for colonization by AMF using a Trypan Blue dying protocol. We first put small diameter (~1mm) roots into cassettes, and cleared the root cells in 10% KOH solution, left for 2-4 days. Then, roots were acidified in a 3% HCl solution overnight, rinsed, and then left in the Trypan Blue dye solution overnight or longer. After dying, roots were destained in dionized water overnight, mounted on microscope slides, and quantified. Using 30 intercepts, we quantified the presence or absence of AMF hyphae, vesicles, and non-AMF hyphae at each intercept. We quantified a total of 20 samples (with 30 intercepts each) from each field site.

**Results**

**Overall Effects**

The ANOVA performed for this experiment showed many of the factors measured significantly affected plant growth and fitness (Table 2). While significant factors varied by measure of fitness, overall both factors (soil type, plant type, microbes) and interactions between these factors were highly significant. This experiment was very robust and had sufficient statistical power to detect the effects of the factors we manipulated.

**Colonization**

We showed that both DUN and IM roots were colonized with AMF in the field, detecting the presence of AMF hyphae, vesicles, and non-AMF hyphae or structures in both field sites (Table 2). Colonization by AMF hyphae is relatively high in both field sites (20.8% at IM and 53.8% at DUN), while colonization by vesicles is considerably lower (13.7% at IM and 11.8% at DUN) at both sites. Non-AMF hyphae and other structures were present in both IM and DUN sites (20.8% at IM and 53.8% at DUN).

<table>
<thead>
<tr>
<th>Site</th>
<th>Percent Colonization by AMF Hyphae</th>
<th>Percent Colonization by AMF Vesicles</th>
<th>Percent Colonization by Non-AMF Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM</td>
<td>.755</td>
<td>.137</td>
<td>.208</td>
</tr>
<tr>
<td>DUN</td>
<td>.290</td>
<td>.118</td>
<td>.538</td>
</tr>
</tbody>
</table>

Table 3. Percent colonization of field roots from IM and DUN field sites. N=20 for each site.

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil Type</strong></td>
<td>1</td>
<td>1</td>
<td>3.4384703</td>
<td>311.0525</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td><strong>Plant Type</strong></td>
<td>1</td>
<td>1</td>
<td>0.0682288</td>
<td>6.1721</td>
<td>0.0135*</td>
</tr>
<tr>
<td><strong>Soil Type*Plant Type</strong></td>
<td>1</td>
<td>1</td>
<td>0.0003042</td>
<td>0.0275</td>
<td>0.8684</td>
</tr>
<tr>
<td><strong>Microbes</strong></td>
<td>8</td>
<td>8</td>
<td>1.6097469</td>
<td>18.2027</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td><strong>Soil Type*Microbes</strong></td>
<td>8</td>
<td>8</td>
<td>0.4752787</td>
<td>5.3744</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td><strong>Plant Type*Microbes</strong></td>
<td>8</td>
<td>8</td>
<td>0.1368769</td>
<td>1.5478</td>
<td>0.1403</td>
</tr>
</tbody>
</table>
### Table 2. ANOVA results for full factorial experiment. Bold values are significant (P<.05).

#### Dependency

We did not find evidence of dependency on AMF in either DUN or IM plants. Instead, both plant types experienced growth and reproductive fitness costs when associated
with AMF. Aboveground biomass was significantly reduced in plants grown with AMF (Figure 7A), and plants grown in sterile soil had the largest aboveground biomass. Belowground biomass was dependent on mycorrhizal status (Figure 7B), while total biomass was lowest in mycorrhizal plants (Figure 7C). Plants grown with mycorrhizae were found to produce less potential fruit (flowers & buds) and flower later than those grown without mycorrhizae (Figure 8).

When we examined differences between DUN and IM plants in dependency on AMF across measures of fitness, we found that DUN and IM responded relatively the same to mycorrhizal status in measures of growth, but not reproductive fitness. Both DUN and IM plants grown with mycorrhizae (M) have lower biomass than those grown without (NM; Figure 9A). Belowground biomass is not affected by mycorrhizal status in either plant type (Figure 9B). DUN and IM plants grown with AMF also see a reduction in overall biomass (Figure 9C). DUN plants, but not IM plants, flowered later when grown with mycorrhizae than without (Figure 10A), while IM plants, but not DUN plants, produced considerably more potential fruits when grown in sterile soil than either mycorrhizal or non-mycorrhizal treatments (Figure 10B).

**Figure 7. Effect of mycorrhizal status on growth.** Across plant types, mycorrhizae (M) are associated with a cost to aboveground biomass (A) and total biomass (C), while belowground biomass (B) is not affected by mycorrhizal status.
Mycorrhizal status is significantly lower for plants grown with mycorrhizae (M) than without (NM).

**Figure 8.** Effect of mycorrhizal status on reproductive fitness. Across plant types, M. guttatus produces the most potential fruit (flowers & buds) when grown in sterile soil (A). Mycorrhizae (M) are associated with later flowering time (B).

**Figure 9.** Effect of mycorrhizal status on growth by plant type. The aboveground biomass (A) of both DUN and IM plants respond the same to mycorrhizal (M) versus nonmycorrhizal (NM) treatments. Both experience a fitness cost when associated with mycorrhize. Neither DUN nor IM belowground biomass (B) was affected by mycorrhizal status. The total biomass (C) of both DUN and IM was significantly lower for plants grown with mycorrhizae (M) than without (NM).

**Figure 10.** Effect of mycorrhizal status on reproductive fitness by plant type. Days to flower (A) was not dependent on mycorrhizal status in IM plants, but DUN plants grown with mycorrhizae (M) flowered later than those grown without (NM). The number of potential fruit (B) of DUN plants was not dependent on mycorrhizae, but IM plants grown in sterile soil produce significantly more potential fruit than those with mycorrhizae and other soil biota (M, NM).
Local Adaptation
We did not find evidence to support local adaptation to native soil fungal communities in either plant type. However, there were some differences in how DUN and IM plants interacted with different inoculum types. Aboveground biomass of both DUN and IM plants seemed to respond the same across inoculum types, with both plant types growing in IM inoculum having the lowest aboveground biomass, while there was no significant difference between other inoculum types (Figure 11A). While IM belowground biomass did not respond differently to different inoculum types, DUN belowground biomass increased when grown with GEN inoculum, even compared to sterile inoculum, which has been associated with the highest measure of growth in other comparisons (Figure 11B). In both plant types, IM inoculum was associated with lower total biomass. Days to flower was not affected by inoculum type in either plant type (Figure 12A). However, IM plants, but not DUN plants produced fewer potential fruit when grown with IM inoculum than when grown with any other inoculum type (Figure 12B). Differences in fruit number between plant types is a result of ecotype-specific traits, not mycorrhizal treatments.

Figure 11. Effect of mycorrhizal inoculum type on growth. Aboveground biomass (A) of DUN and IM plants show respond the same to inoculum origin, with IM inoculum corresponding with significantly lower aboveground biomass than other inoculum types. Belowground biomass (B) of IM plants is not affected by mycorrhizal origin, while DUN plants grown with GEN inoculum have higher belowground biomass. For both plant types, total biomass is decreased in plants grown with IM inoculum.
Effect of Soil Type

Figure 12. Effects of mycorrhizal inoculum type on reproductive fitness. Days to flowering (A) was not dependent on mycorrhizal inoculum type in either plant type. For DUN plants, number of potential fruit (B) was not dependent on mycorrhizal inoculum type, while IM plants produced significantly less fruit when grown with IM inoculum, and the most fruit when grown with sterile inoculum.

Figure 13. Effect of soil type on number of potential fruit and number of flowers. IM, but not DUN plants produce significantly (~65%) more potential fruit than when grown in IM soil than when grown in DUN soil (A). In DUN soil, number of flowers is not dependent on inoculum type, while in IM soil, plants grown in IM inoculum produce significantly fewer flowers than those grown in other inoculum types (B).

We found a strong effect of soil type on both growth and reproductive fitness, where both plant types had significantly higher fitness in IM soil versus DUN soil (P values by fitness measure). We also found that soil type strongly affected fruit number in IM plants (Figure 13A), but not DUN plants. For plants grown in DUN soil, number of flowers is not dependent on inoculum type, while plants grown in IM soil exhibit different flower numbers depending on what type of inoculum they are grown in. Plants grown in IM inoculum produced the fewest flowers, while plants grown in sterile inoculum produced the most flowers.
Specificity
The data for this section is currently a work in progress. Sequencing will be completed during the summer of 2017, and this data will be subsequently added as it becomes available. We hope that this data will help to explain some of the patterns seen above.

Discussion
Dependency
Neither DUN nor IM plants were dependent on AMF in the way we expected. IM plants, because there may be some cost to associating with AMF for short-lived annuals, were not expected to show dependency on AMF, which they did not. However, we did not expect that IM plants would actually exhibit some cost to associating with AMF. This may suggest that perhaps associating with AMF is not a choice for IM plants, which would possibly be better off if able to avoid associating with AMF to begin with. DUN plants, in contrast, were expected to benefit from AMF, as they are a long-lived perennial living in an extremely nutrient poor environment. However, the results of this experiment showed that DUN plants also experienced a cost when associated with AMF. My experiment ran in the greenhouse long enough for both plant types of full manifest their differential growth forms, and previous experiments that did detect dependency (Klironomos, 2003) ran for a similar time period. It is possible, however, that DUN plants could show positive effects of associating with AMF over their lifetime if grown for multiple years in the greenhouse.

Local Adaptation
Neither DUN nor IM plants showed local adaptation to native AMF communities. There was some variation in response to AMF by IM plants across inoculum types, with IM’s home IM inoculum being the costliest to growth and reproduction for both plant types. This could suggest some kind of negative feedback effect of home soil inoculum. DUN plants also reacted most negatively to IM inoculum, but are very closely related to IM plants, so both may also be susceptible to the type of pathogens present in IM inoculum. Previous work (Klironomos, 2000) suggest that AMF are not host specific. The large number of plant host species (~300,000) and the small number of fungal symbiont species (~150), as well as the results of this study, supports this generalist model of the AMF-plant symbiosis, where local adaptation is unlikely to evolve. This lack of host-symbiont specificity has yet to be explained, as many highly stable symbioses (of which the AMF-plant symbiosis is one) are highly specific.

Effect of Soil Type
Soil conditions are known to strongly affect both AMF and plant fitness, so it is no surprise that we saw such significant effects of soil type on plant growth and fitness in this experiment. Our results suggest that soil type affects how plants interact with the microbial community, with microbial origin being important in IM soil, but not DUN soil. The abiotic conditions of DUN soil, basically sand, may supersede the effects of plant-AMF interactions, putting all plants on a level playing field in these difficult soil conditions.
References


