

Making friends with Fungi

How does mycorrhizal symbiosis affect plant growth?

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Background research

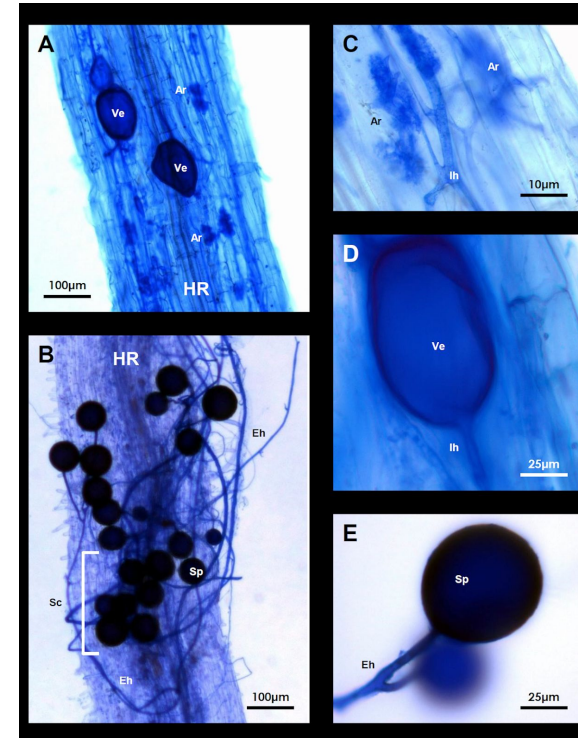
Mycorrhizal Symbiosis (Latin) = Mushroom-Roots Living Together

“The roots of most plants are colonized by symbiotic fungi to form mycorrhiza, which play a critical role in the capture of nutrients from the soil and therefore in plant nutrition.”

Smith, S.E., & Read, D.J. (2008).

Arbuscular mycorrhizae (AM) are a type of mycorrhiza in which the symbiont fungus (Arbuscular mycorrhizal fungi, or AMF) penetrates the cortical cells of the roots of a vascular plant forming arbuscules. Both paleobiological and molecular evidence indicate that AMF are an ancient symbiosis that originated at least 450 million years ago. Arbuscular mycorrhizae are believed to form associations with 85% of all terrestrial plants.

Willis, A., Rodrigues, B. F., & Harris, P. J. C. (2013).

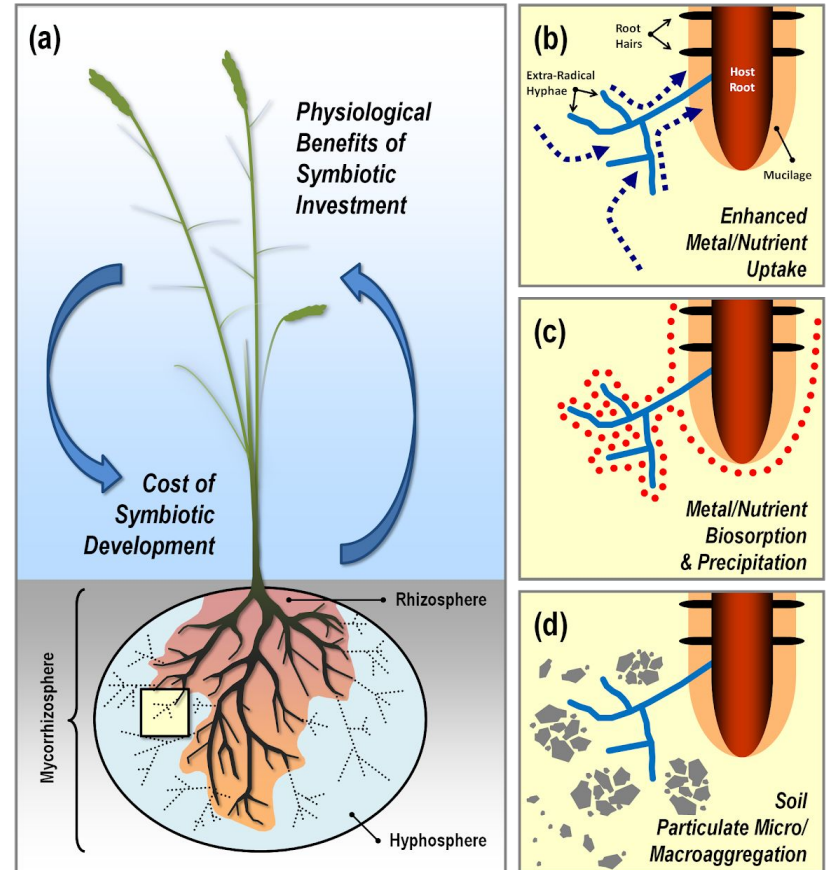


How it works

The mycorrhizosphere plays an important role in plant nutrition, stress tolerance (drought, metal toxicity) and soil microbiology.

There is a cost for maintaining this symbiotic relationship. Plants exchange sugars (glycogen) with the fungus for nutrients and water. There is a trade off between plant growth and development of the mycorrhizal symbiosis.

Audet, P. (2012).



Testable question

**How does mycorrhizal symbiosis
affect plant growth?**

I tested this question by growing plants under
different soil conditions.

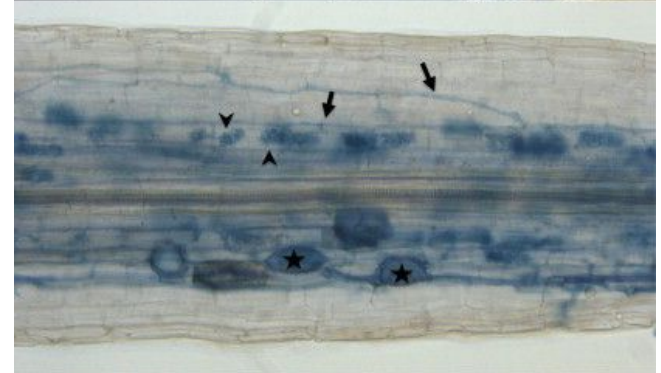
Study Design

Rhizophagus irregularis & Funneliformis mosseae

Rhizophagus irregularis (previously known as Glomus intraradices) and Funneliformis mosseae (previously known as Glomus mosseae) are arbuscular mycorrhizal fungi most commonly used as a soil inoculant in agriculture and horticulture. These AMF species are also the most commonly studied research species; the fungal inoculum is even available commercially at garden stores.

Project Experimental Design:

The goal of this project is to explore the effects of mycorrhizal symbiosis on plant growth. Rhizophagus irregularis & Funneliformis mosseae were selected as the study species because (1) easily available and (2) easily grown indoors under greenhouse conditions. Also, other people have studied mycorrhizal symbiosis using these species. So there is available information for comparison of results.



Hypothesis

If I add mycorrhizal fungi to the soil, this will result in colonization of plant roots and formation of mycorrhizal symbiosis.

Plants colonized with mycorrhizal fungi (Myc+) will grow better than non-colonized plants (Myc-).

Materials for Greenhouse Experiment

- Plant pots
- Potting soil without arbuscular mycorrhizal fungus (Myc-)
- Potting soil with arbuscular mycorrhizal fungus (Myc+)
- Sand
- Barley seeds
- Measuring utensils



Variables

Manipulated	Responding	Controlled
<p data-bbox="63 437 320 489">Types Soil:</p> <p data-bbox="63 528 529 579">Sand vs. Potting Soil)</p> <p data-bbox="63 619 537 729">With or Without Mycorrhizal Fungus:</p> <p data-bbox="63 768 378 820">Myc+ vs. Myc-</p>	<p data-bbox="672 437 958 489">Plant Height</p> <p data-bbox="672 539 1112 656">Plant Biomass (Dry Weight)</p> <p data-bbox="672 706 1083 751">Root Colonization</p>	<p data-bbox="1275 437 1841 489">Amount of Soil in the pots</p> <p data-bbox="1275 539 1731 645">Amount of Water per week</p> <p data-bbox="1275 706 1707 757">Amount of Sunlight</p> <p data-bbox="1275 804 1638 856">Growing Period</p>

Controlling Variables

Why should it be controlled and how it can be controlled?

Amount of Soil in the pots: More soil means more space for roots to grow and in turn more mycelium. This can be controlled by measuring out the same amount of soil for each plant.

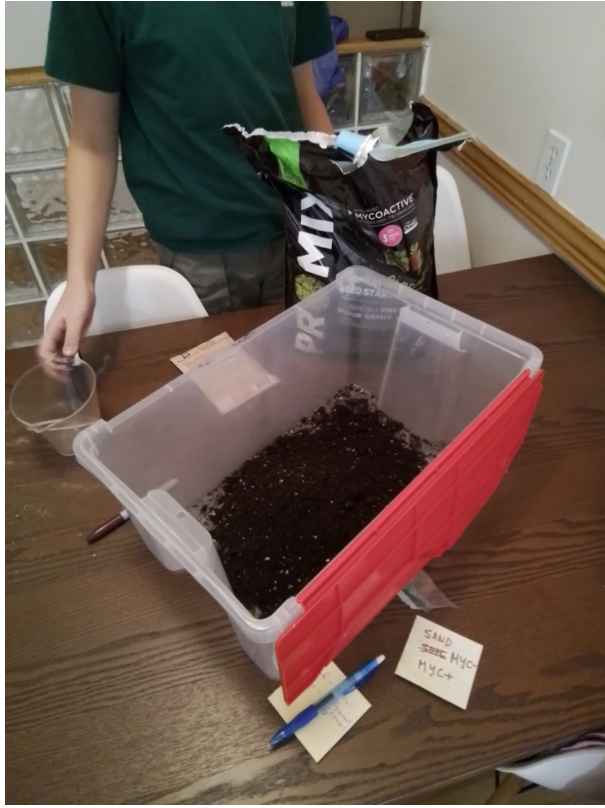
Amount of water per week: A plant getting more or less water could affect the plants growth. This can be controlled by measuring the same amount of water for each plant.

Amount of sunlight: A plant getting more or less sunlight could affect the plants growth, because of this the data could be wrong. This can be controlled by setting the growing lights to the same consistency.

Setting Up the Greenhouse Experiment

Planting Procedure

1. Mix Soil Substrates in a Bassin
 - Sand (100% Sand)
 - Myc- (50% Sand, 50% Potting Soil without Mycorrhizal Fungus)
 - Myc+ (50% Sand, 50 Potting Soil with Mycorrhizal Fungus)
2. Label all pots clearly.
 - Sand 4x Pots
 - Myc- 4x Pots
 - Myc+ 4x Pots
3. Add soil to pots (300ml soil per pot)
4. Water all pots (250ml per pot)
5. Poke 3x holes (1cm depth) per pot (in a triangle shape)
6. Add 3x seeds per hole (9x seeds per pot)
7. Take lots of pictures
8. Grow plants in greenhouse for 5x weeks



Soil Mixing



Seeding



Watering



Growing Plants (Week 1)



Growing Plants (Week 5)

Harvesting the Plants

Procedure

1. Take plants carefully out of pots.
2. Gently remove soil from roots.
3. Separate roots and shoots.
4. Take a sample of roots by cutting off a portion of the outer roots and placing in a water filled container.
5. Place in bags and label them the same as what you labeled your pots.
6. Dry plants by placing them in the oven and heating for 40 minutes at 350 degrees.



Assessing Root Colonization

Root Staining: Ink and Vinegar Method

[From: Vierheilig et al. (1998) in Appl Environ Microbiol]

Materials:

- Fresh plant material (roots)
- Household vinegar
- Liquid black ink
- Hydrogen peroxide
- Glycerin
- Fresh water
- Plastic Containers (or Phials)

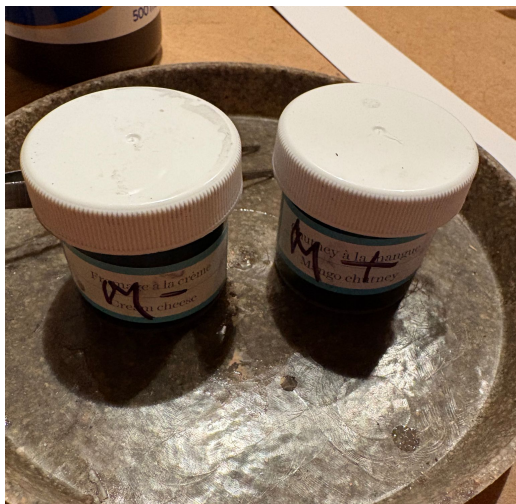
Root Staining

Procedure

1. Collect fresh root subsamples (approx. 2cm) from plants grown in the Myc- and Myc+ treatments
2. Rinse clean all samples and combine all subsamples into two separate containers (Myc- and Myc+)
3. Add hydrogen peroxide to both containers; close containers and warm solution in a bain-marie
4. Make staining solution by combining vinegar and ink in a separate container; warm solution to help combine these substances
5. Carefully rinse roots from the hydrogen peroxide, and add staining solution to root sample container; allow up to 1 day to soak in solution
6. Carefully rinse roots from the staining solution using vinegar and then fresh water
7. Add glycerin to the stained roots to preserve until root microscope slide preparation.



Staining Roots ↑



↑
Rinsing Roots



←
Preserving
Roots

Measuring Plant Growth

Materials

- Scale
- Tape measure
- Dried shoots
- Dried roots
- Recording table

Weighing

1. gather dried plants
2. separate roots from shoots
3. weigh roots by placing on the scale and recording on data sheet
4. lay shoots flat and measure height by using a tape measure
5. weigh shoots
6. record measurements



Preparing Microscope Slides

Materials

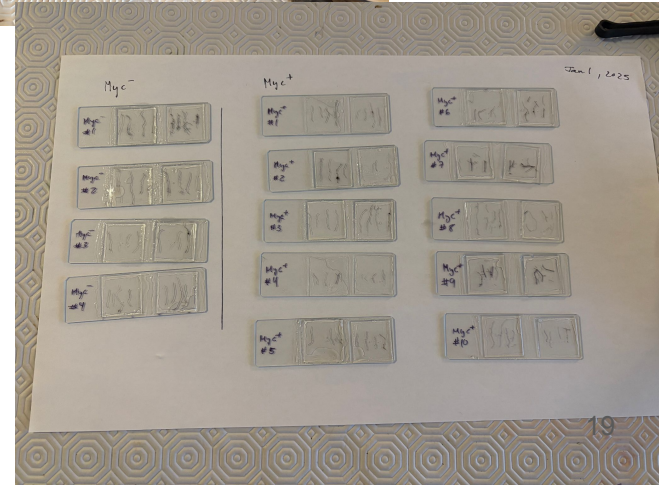
- Microscope
- Microscope Slides (Glass) and Slide covers
- Petri Dish
- Pipette (eye dropper)
- Dissection Tools (Tweezers, Scissors, Probes)
- Clear nail polish

Root microscope slide preparation

1. Label all microscope slides with soil treatments (Myc- or Myc+)
2. Transfer stained root samples to Petri dish
3. Use dissecting tools to pull apart the roots and cut into smaller pieces (up to 5-10mm long)
4. Place 8 root pieces onto each microscope slide
5. Using the pipette, add a small amount of the glycerin solution on to the roots
6. Cover the root pieces with the slide covers
7. Seal around the slide covers with clear nail polish; allow to dry and touch up if necessary.

For the Myc- treatment, 4x slides were prepared using a total of 32 root pieces

For the Myc+ treatment, 10x slides were prepared using a total of 80 root pieces.



Results

- **Plant Growth**
 - **Average number of plants**
 - **Root biomass (dried)**
 - **Shoot biomass (dried)**
 - **Plant heights**
- **Root Colonization (Microscope Analysis)**
 - **Myc+ Root fungal colonization**
 - **Myc- Root fungal colonization (Control)**

Results: Root Colonization

Microscope slides myc +

Slide 1: 1 fungal structure (/10 root segments)

Slide 2: 0 fungal structure (/10 root segments)

Slide 3: 1 fungal structure (/10 root segments)

Slide 4: 0 fungal structure (/10 root segments)

Slide 5: 2 fungal structure (/10 root segments)

Slide 6: 2 fungal structure (/10 root segments)

Slide 7: 0 fungal structure (/10 root segments)

Slide 8: 1 fungal structure (/10 root segments)

Slide 9: 1 fungal structure (/10 root segments)

Slide 10: 3 fungal structure (/10 root segments)



11 fungal structure / 100 root segments = approximately 10% root colonization frequency

Observed fungal vesicles (that look like balloons) and hyphae (that look wires).

Results: Root Colonization (Control)

Microscope slides myc-

Slide 1: 0 fungal structures (/10 root segments)

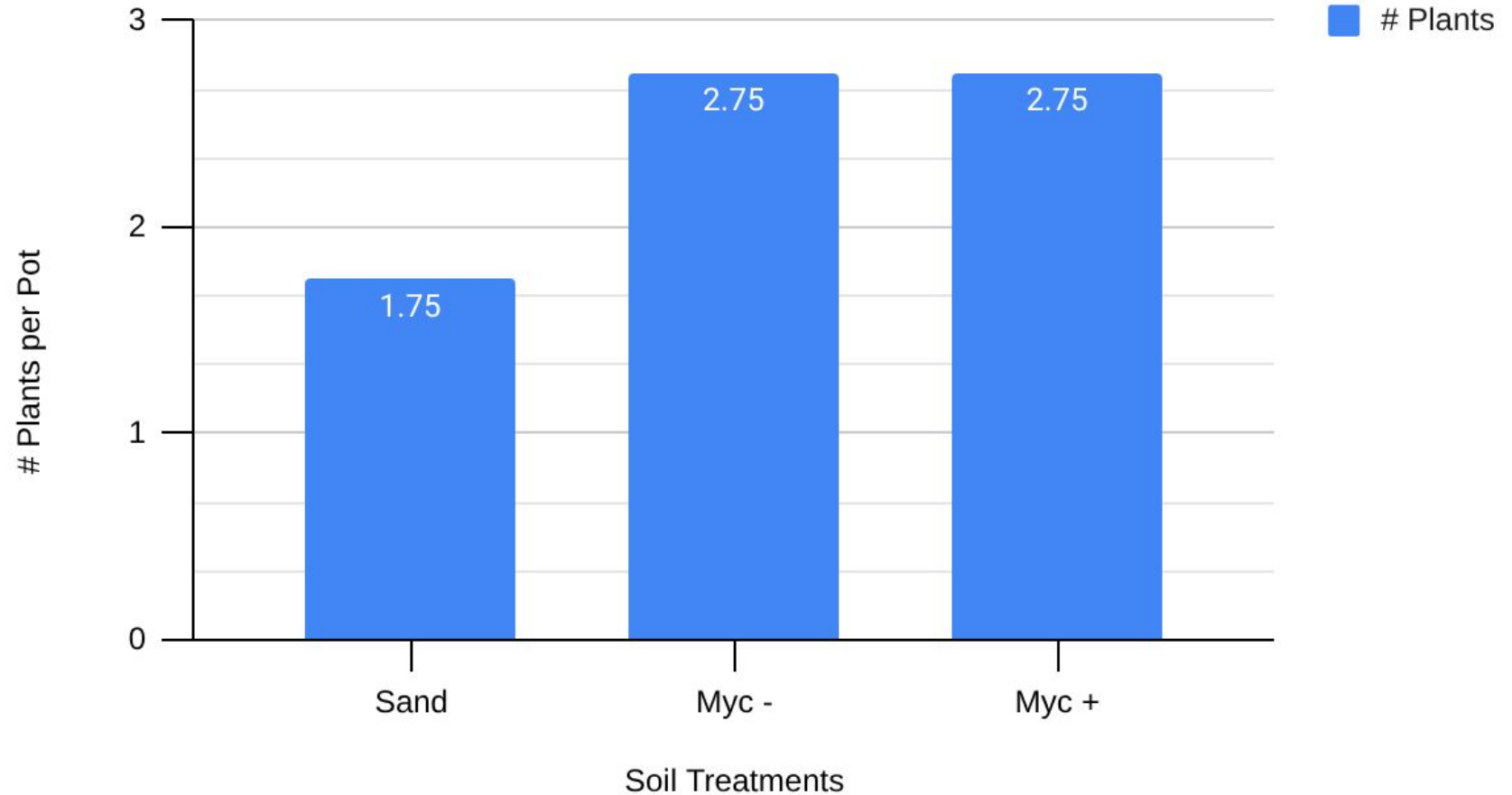
Slide 2: 0 fungal structures (/10 root segments)

Slide 3: 0 fungal structures (/10 root segments)

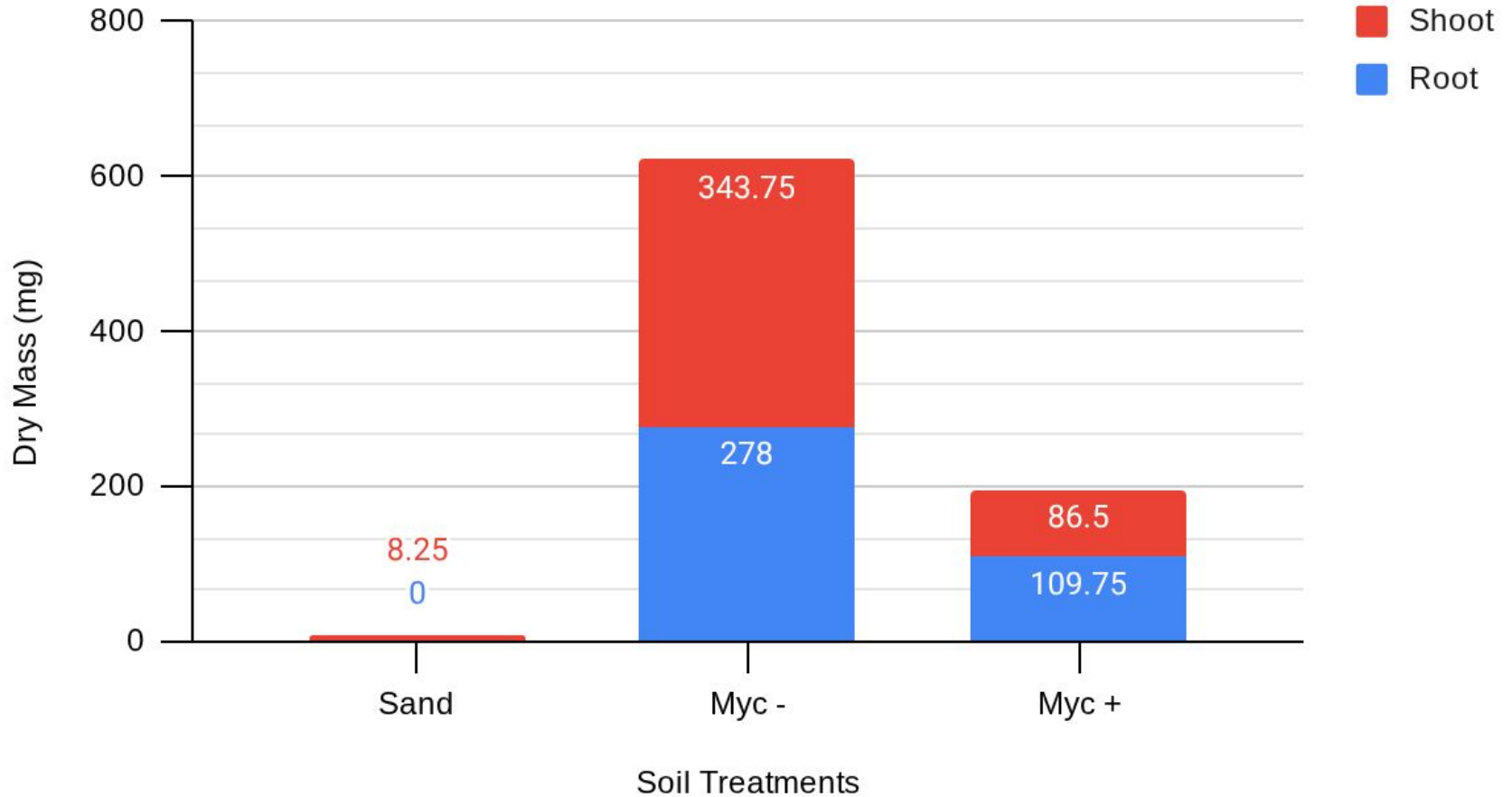
Slide 4: 0 fungal structures (/10 root segments)

0 fungal structure / 40 root
segments = no root colonization
(as expected)

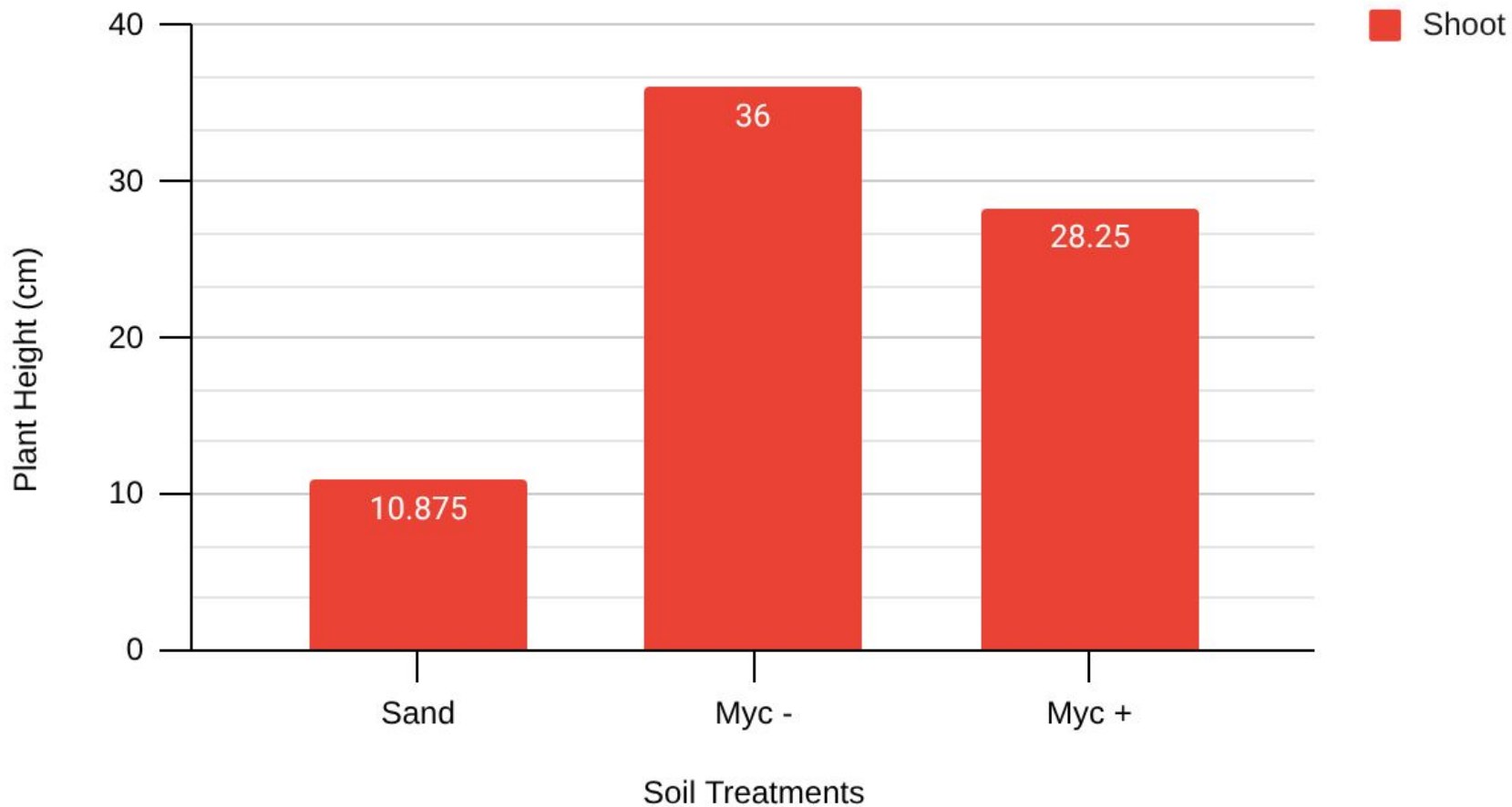
Average # Plants per Pot



Total Plant Dry Mass



Maximum Plant Height



Analysis

The manipulated variables in this experiment were sand, potting soil without mycorrhiza (Myc-), and potting soil with mycorrhiza (Myc+). The responding variables were the plant height, dry weight, and mycelium root colonisation.

On the graphs you can see that Myc- surprisingly had the best growth over all. This is interesting because Myc+ had microbial colonisation. The mycorrhizal symbiosis was expected to help the plants grow. But there is a trade off, in which the plant gives nutrients to the mycorrhizal fungus and the mycelium acts as a root extension, probing new areas of soil to better the plants growth and nutrient intake, by absorbing larger pieces of nutrients found in the soil.

Conclusion

Hypothesis: Plants colonized with mycorrhizal fungi (Myc+) will grow better than non-colonized plants (Myc-).

This hypothesis was not supported by my data:

My experiment was about growing plants in different soil conditions. I thought that Plants colonized with mycorrhizal fungi (Myc+) will grow better than non-colonized plants (Myc-).

Surprisingly Potting soil without mycelium (Myc-) grew the best overall. The potting soil with mycelium (Myc+) did not grow as well. This is interesting because plants with mycelium generally do better than plants without it. the reason plants with it do better is because of symbiosis, the mycelium in the soil bonds with the plant and acts as a root extension, this is a trade off as the plant gives the mycelium nutrients, and the mycelium breaks down bigger pieces of nutrients in the soil, which the plant absorbs and the cycle continues.

Next steps

My hypothesis was not supported by my data as the Myc- plants grew better than the Myc+ plants. In the future to improve this experiment I could grow more plants and grow them under different conditions to see what would happen.

Real world connections

My experiment was to explore the effects of mycorrhizal symbiosis on plant growth. In the real world, we use chemical fertilisers to grow plants. But chemical fertiliser is expensive, and using too much is not good for the environment. A more eco-friendly solution would be to use mycorrhizal symbiosis to help plant growth and produce more food.

This technique has become so common that you can even buy mycorrhizal fungi in garden stores. More farmers using mycorrhiza means less chemical fertilizer which would be more eco-friendly. But what about other natural fertilisers like cow or animal manure? This can have the same effect, but you have to reapply it every year. Mycorrhizal networks only have to establish themselves once and will help plants grow better over the long term.



Sources of error

Potential sources of error in this study include:

1. Plants were grown for 5x weeks. Maybe they needed more time to grow. If plants were grown for longer, maybe the mycelium could have better helped the plants.
2. Only 4x pots per treatment were grown. Maybe more pots (replicates) would be better.
3. The root staining could have been better. Differentiating the fungal structure was difficult.
4. The microscope that was used was basic. I did my best, but it was difficult to focus the image at higher magnification.

Citations

Audet, P. (2012). Arbuscular Mycorrhizal Symbiosis and Other Plant–Soil Interactions in Relation to Environmental Stress. In: Ahmad, P., Prasad, M. (eds) Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. Springer, New York, NY.

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Acknowledgments

I worked on this project with my dad who studied arbuscular mycorrhizal symbiosis from 2006-2013.

We worked together on the background research, study design, data collection and analysis.

