



## Effect of Mycelium on Different Native Tropical Plant Seed Germination Rates

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### Introduction

Deforestation across the Amazon Rainforest is a huge concern, with over 20% of the rainforest having been lost to accommodate practices of agriculture, raising cattle, and gold mining. (Lindahl et al., 2002) This experiment proposal will be executed for the purpose of finding a sustainable practice regarding natural fertilization for agriculture, improved reforestation methods, and even possible economic benefits for locals in and around the Peruvian Amazon. The goal result of this specific experiment is to test how mycelium infused soil affects seed germination vs seeds that germinate in non-infused soil.

Mycelium are the rootlike structures used by fungi to obtain nutrients and water. The hyphae of the mycelium secrete enzymes important for breaking down raw and organic material REF. Companies have produced excellent results of utilizing mycelium for different products, such as fully biodegradable plastic, cloth, and other materials. Mycelium is incredibly easy to produce, it does not require much water to thrive, and it does not damage the surfaces it grows on, unlike other agricultural products. (Perez-Moreno & Read, 2000) This makes cultivation rather inexpensive and more sustainable than other farming.

Mycelium has been tested over the years to look closely at its benefits. It has shown to

offer more nutrients to surrounding plants, like nitrogen and phosphorus. Fungi positively influences soil fertility for other plant use, promotes plant health, and provides nutrients to plants as a result of their organic material cycling. (Finlay, 2008) Many soil scientists agree that, even in agricultural soils, mineralization of nitrogen and phosphorus involves a sequence of processes for which the living fungi biomass provides the enzymes and for dead organic material, much of the substrate. (Perez-Moreno & Read, 2000) Fungi and surrounding plants have a mutually beneficial relationship. The fungi facilitate increased transfer of soil nutrients to the plant. It does this by attaching to the roots of the plant and acting as an extension of the root system. In return, the plant provides carbon and carbohydrates to the fungi. (Yao et al., 2001) This relationship should show prosperity to both parties. There are many studies that focus on explaining this relationship. Fungi contains ligases and polyphenol oxidases, so they are expected to contribute in a major way to decomposition as well as to plant nutrition. (Perez-Moreno & Read, 2000) These decomposition and nutrition aspects are what makes fungi so attractive to plants who need assistance gathering essential components.

## Methods

### *Study Area*

This experiment was conducted at Finca Las Piedras (S 12° 13.502, W 069° 06.649), the Alliance for a Sustainable Amazon's research center in Madre de Dios, Peru.

### *Experimental Design*

Part 1: Collecting Fungi to utilize mycelium.

Along manmade trails at the Finca Las Piedras research station in the Peruvian Amazon (terra firme forest), Madre de Dios region, fungi of similar families were collected to be identified.

Fungi growing out of the ground was selected, rather than fungi growing on other surfaces. This was done because the experiment requires fungi to thrive in soil.

Collection was done using rubber gloves and carefully picking up and placing the fungi into plastic bags. Fungi was picked very gently and carefully to also obtain any root that was within the soil. 100 mushroom fungi were picked.

Fungi was observed and researched for identification. Identification occurred in the lab only a few minutes after collection, and the fungi was stored in plastic bags. Identification is done for control purposes within the experiment, as not every kind of fungus behaves the same.

Part 2: Soil Preparation.

Soil was obtained from an area in the rainforest that appeared to be fungi/mycelium free. The soil was analyzed and lacked visible fungi and other vegetation.

The soil filled a bed 1 meter by 3 meters, and 12 small bags. The beds were filled with soil and packed down; the bags were packed semi-dense with soil to prevent root rot.

The soil was halved, and the fungi was

incorporated into one half (half of the bed and 10 bags). 100 mushrooms were incorporated in a ½ cubic meter of soil.

A physical barrier of wood was put in between the two sides of the bed to keep the mycelium soil apart from the other soil. This barrier must be at least 25 cm down into the soil to keep the soils separated for control within the experiment.

Moisten the soil with water to prepare for the seed planting. If the soil is too dry, the seeds will likely not sprout at all.

Part 3: Selecting/obtaining seeds.

Seeds of 3 different plants were obtained at a market in Puerto Maldonado, as time was a limiting factor so gathering seeds from the forest was not an option.

For this experiment, 20 of each of the three kinds of seed is necessary. This number of seeds was chosen because it allows for enough varied observation, without taking up so much space (space was another limiting factor).

Papaya, lettuce, and passion fruit were selected because papaya and passionfruit are native to the region, and all three have germination times of less than 2 weeks.

Short germination was a key factor for this experiment as the time needed to perform this experiment from start to finish needed to be within 3 weeks.

Part 4: Planting the seeds.

Passion fruit seeds were planted in the bags. Papaya and lettuce seeds were planted in the bed.

Bags were about 8.5cm in diameter and 20 cm in height. The soil was packed in relatively dense.

The bed was divided into four sections. First, split in half with the barrier to divide mycelium soil and regular soil. Then 10 rows were made across the whole bed, 5 rows on each side. The rows were 24 cm apart.

5 seeds of papaya were planted in a row, for 2 rows (10 seeds total) on each side. The

same was done with the lettuce but for 3 rows (15 seeds total) on each side.

Place individual seeds about 1.5 cm deep, and lightly cover with a layer of dirt. For the passion fruit place 1 seed 1.5cm deep in each bag and lightly cover with dirt.

Label the sides of the bed mycelium and regular. Label which rows are lettuce, papaya, and passionfruit. In the bed, each seed planted was marked for precise monitoring.

Part 5: Observing the progress.

Observation is occurring for 14 days after planting.

Each day after planting, look for sprouts. While some sprouts may look different, it is important to watch for anything breaching the surface of the dirt so it can be monitored.

Keeping the soil moist for the seeds is essential. Shade can help with this if the soil is drying too quickly.

Once sprouts are visible, take photos each day. Organize the photos to remember which sprouts are in which photos. Photos are helpful for visually comparing day-to-day change.

Compare growth progress, rate, and success of the seeds in the mycelium soil to the seeds in the regular soil. Ex: which sprouted first; which seedlings grew taller; if any did not sprout at all; etc.

Keep observations of time(days), size (width of stem), height(cm), number of leaves, color, and health in a spreadsheet or chart to monitor progress and change.

*Found Fungi ID for the infused soil.*

Leucocoprinus (30 mushrooms)

Coprinellus (70 mushrooms)

100 total mushrooms mixed with 1 cubic meter or soil.

## Results

On the final day of data collection for the experiment (day 13), one lettuce seed sprouted. It sprouted on the side of the mycelium-infused soil. Germination was unsuccessful in all, but the one lettuce seed planted. As there is not concise data to support or deny the hypothesis, the results are inconclusive. It is impossible to determine whether the mycelium was helpful or hurtful to the germination of the seeds.

## Discussion

There are several possible outside factors that could've negatively affected the germination of the seeds planted. The third day after planting the seeds, a friaje (cold winds blowing up from Antarctica) was present for four days.

A Friaje is a natural event that occurs in Peru in the months of June, July, and August. It is caused by winds blowing upwards from Antarctica. Temperatures less than 10 ° C to 20 ° C and is usually accompanied by dry air. (Montes and Pedro, 2016). An extreme friaje is classified as anything below 15° C. The temperatures experienced during the friaje at the Finca Las Piedras research station during the time of this experiment were between 10.5° C and 14° C.

Temperature and rainfall data is gathered each morning on site by our resident research team. The fourth day after planting was a high of 28°C, a low of 12.5°C, and received 25cm of rain. The fifth day was a high of 13°C, a low of 11°C, and 8.5cm of rain. The sixth day was a high of 14°C and a low of 11°C. The seventh day was a high of 18°C and a low of 13.4°C. During these days, the daily sun recommendation (4-6

hours) was not met, and the temperatures were significantly lower than necessary (15.5C-32C), as per the information provided on the supplied seed packaging. Although temperatures and sunlight for the first three days after planting were adequate, the conditions did not persist through what would've been the entire germination process. Consistency with temperature, sunlight, and rainfall was not present during the project time. Whole plant performance for the papaya, regarding growth and fruiting, is affected and greatly limited by cold conditions below 11°C. Papayas germinate/grow best in temperatures over 20° C, during only 4 months of the year when subtropical areas are humid and hot enough. (Allen 2002)

Seeds are especially vulnerable to 'chilling' injury at temperatures less than 10-15°C during their germination periods. (King and Roberts, 1979; Chin, 1988) The adult plants of these species (papaya, passion fruit, lettuce) also struggle to withstand these cold temperatures. Passion fruit plants are prone to cold injury below 18°C because they are very sensitive to lower temperatures. (Tripathi 2018) While lettuce is accustomed to growing in cooler temperatures when compared to papaya and passionfruit, it has been known to struggle in difficult conditions. (Mou, 2008) Studies have shown that the optimum climate for lettuce seed germination is above 18.5°C. (Lee et al, 2015)

A mesh bird net was not utilized to cover the bed or the bags. This left it vulnerable to wildlife at most times of the day and all of the nighttime. Possibilities of predators for the seeds could be birds, domestic cats living on the property, or other small rodents that live in the vicinity (mice and rats).

#### Method improvements

To improve this experiment there are

several aspects that can be altered in terms of controlled variables and possible outside impacts.

Adding a mesh cover over the bed would protect the seeds from most birds and could've deterred other animals from taking the seeds. This would also allow integrity for another outside variable to be put under control.

Using grow/heat lights for days with less sun and cooler temperatures can give the seeds the necessary environment to germinate and thrive. Another similar option in this category would be utilizing a greenhouse to allow for control on these other experimental variables (sunlight, heat, and protection from animals).

Giving more time for the project would've allowed multiples attempts, even for different times of year, as some seeds naturally germinate only certain points of the year. This also would be beneficial as 9 months of the year friaje does not occur. (Montes and Pedro, 2016)

More seeds(quantity)/Larger variety of native seeds. This would eliminate the question of whether the seeds used were at fault. If they had been stored improperly or were very old this could've had an impact on their germination. (Ellis et al, 1991)

Taking temperature readings of the soil throughout the germination period to ensure the seeds are in a range of possible growth.

Overall, this experiment ended with inconclusive results in terms of the mycelium-infused soil germination. Since it is not known exactly what caused the seeds to fail, there is room for improvement on multiple levels to attempt to control these possible variables.

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