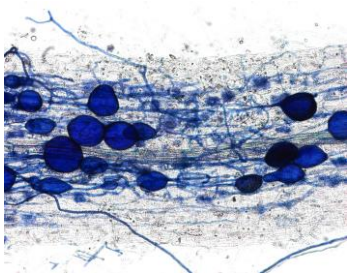
Effect of soil conditions on the mycorrhizal colonization of *Cannabis sativa*



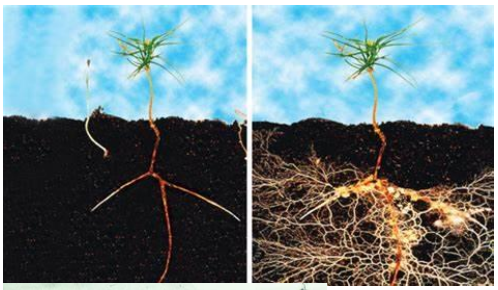


Lyndsay Rayner
Supervisor: Dr. Karen Christensen-Dalsgaard

Hello, my name is Lyndsay Rayner, and I'm going to present to you my research on the effect of soil conditions on the mycorrhizal colonization of Cannabis sativa

BACKGROUND – Arbuscular Mycorrhizae[10,13]

- "AMF"
- Symbiosis between fungus and plant roots
- Typically mutualistic
- Agriculture applications



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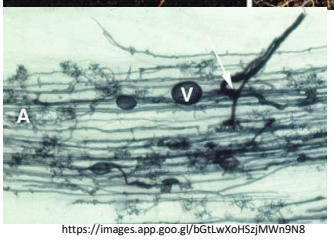

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- Arbuscular Mycorhhizal fungi (or AMF) is a type of symbiotic fungi that form relationships with the roots of almost 80% of plants on earth.
- The fungi penetrate the root cells and establish arbuscles for nutrient transfer, and vesicles for storage (which can be seen as the dark spots in the bottom image).
- This relationship is often mutualistic as the fungi increases the absorptive surface area of roots increasing water and nutrient acquisition for the plant, as well as providing protection against pathogens and other environmental stressors., and in return the plant provides the fungus with necessary carbon-rich photosynthates.
- AMF occur naturally in soils, but are also applied as an agricultural inoculant to improve the health and yield of crops.
- Despite being domesticated and utilized by humans for thousands of years, the interaction between Cannabis plants and mycorrhizae is largely unknown and very understudied.

BACKGROUND – Hemp (*Cannabis sativa)* [2]

- Low THC variety
- Multi-purpose crop
- Rising in popularity

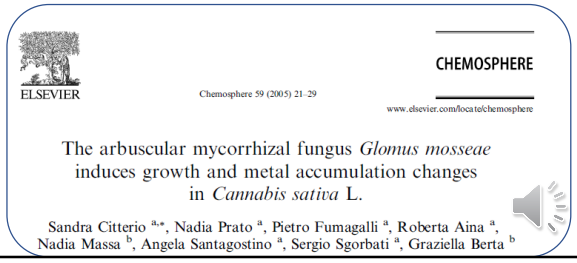
https://www.uky.edu/ccd/sites/www.uky.edu.ccd/files/hemp.jpg

- So this brings me to my plant of study, Cannabis sativa, also known as industrial hemp
- The main distinction between hemp and other sub-species of cannabis, is the low percentage of the psychoactive molecule THC. In order to be considered hemp, the THC content must be less than 0.3%
- Hemp is a multi-purpose crop, with almost all parts of the plant being utilized for a variety of things: edible, nutritious seeds, relatively high in CBD (the therapeutic molecule used in health products), and the fibers from stems and leaves used for textiles and building materials.
- Thanks to its versatility, hemp continues to rise in popularity as a cash crop, and that's why it is important to further investigate the agronomy and ecology of this plant to establish best growing practices.

BACKGROUND – *C. sativa* + mycorrhizae

- Largely understudied
  - McPartland et al. (1997)
  - Zubek et al. (2012)
  - Citterio et al. (2005)
- Mostly non-scientific, anecdotal information

https://steemit.com/weedcash/@choosefreedom/why-mycorrhiza



ELSEVIER

Chemosphere 59 (2005) 21–29

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

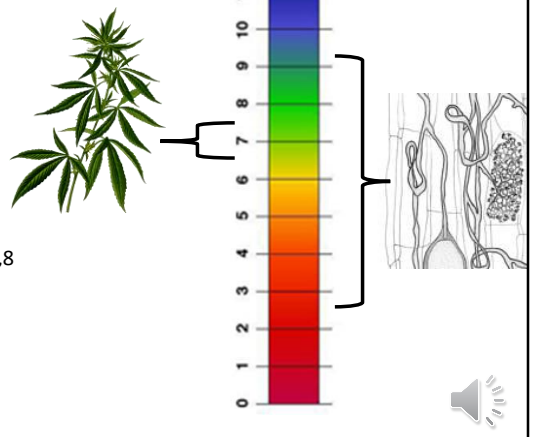
The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L.

Sandra Citterio [a,*], Nadia Prato [a], Pietro Fumagalli [a], Roberta Aina [a], Nadia Massa [b], Angela Santagostino [a], Sergio Sgorbati [a], Graziella Berta [b]

- The knowledge gap on this topic was very apparent During my literature review; only three research papers could be found mentioning mycorrhizae and cannabis together
- McPartland et al. (1997) simply acknowledged the presence of AMF in hemp roots, Zubek et al. (2012) stated colonization was relatively lower in cannabis compared to other medicinal plants, and Citterio et al. (2005) discussed the parasitic effect AMF had on hemp grown in heavy metal contaminated soils
- Beyond these papers, the majority of information regarding mycorrhizae and cannabis is mostly anecdotal, and widely used to promote inoculant products

BACKGROUND – Effects of pH

- Optimal range for organisms [4, 14]
- Availability of nutrients [8]
- Uptake of heavy metals [3]
- Less colonization in acidic soils [4,5,8]



- Many factors may impact the colonization of AMF.. My study attempted to focus on soil pH
- all living organisms have an optimal pH range for survival, depending on the species
    - Since there are over 200 species classified as AMF, we can see it has a much wider optimal range than hemp
- Soil pH is an important variable, because it affects the availability of both necessary nutrients, and toxic heavy metals. Both of which may affect how these two organisms interact with each other.
- Although previous studies looked at different plant species, most concluded there was generally less colonization in acidic or lower pH soils

RESEARCH QUESTION:

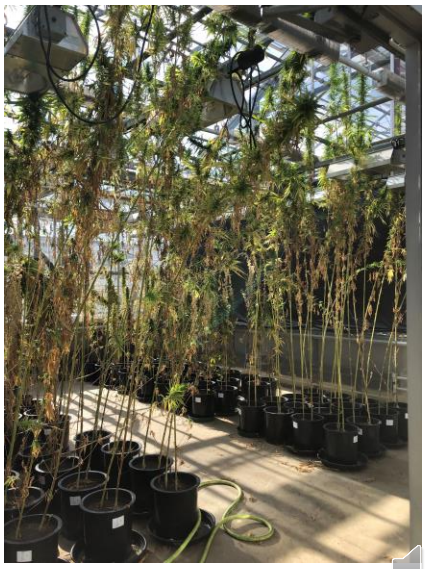
"Does soil pH have an effect on the colonization of AMF on different varieties of hemp roots?"

My research question was: does soil pH have an effect on the colonization of (AMF) on different varieties of hemp roots?"

METHODS - Growing

- Collaboration with InnoTech Alberta
- Natural field soil
- Controlled greenhouse setting

https://innotechalberta.ca/about-us/



- This study was done in collaboration with InnoTech Alberta and their pre-existing experiments using three varieties of hemp grown in different soil conditions
- To begin, the soil was collected from the experimental farm in Vegreville Alberta, from areas “previously determined” to have a relatively “low” or “high” pH value
- Soil was not autoclaved as to maintain naturally occurring microbes, including any potential AMF
- The plants were grown in a controlled greenhouse setting.

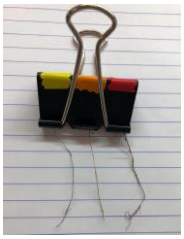
METHODS – Sampling

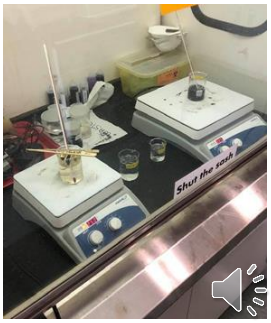
- Roots harvested at 18 weeks
- 3 segments per plant
- Soil sample for pH analysis

- At 18 weeks, I harvested the roots and collected my samples.
- I took 3 segments from each root (top, middle, and bottom, as indicated by the red arrows) this helped to generate an average for the entire pot and to account for potential soil stratification from continuous watering
- I also collected soil samples to conduct a more detailed soil analysis at a later date.

METHODS – Clearing & staining roots

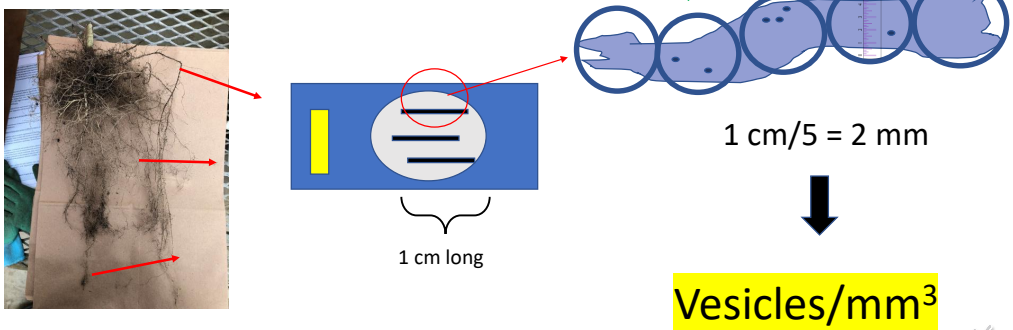
- Clear roots in 10% KOH [9]
- Acidify and stain with lactic acid & Trypan Blue [9,11]
- De-stain with 1:1 lactic acid & glycerol [11]
- Prepare slides in glycerol [6]



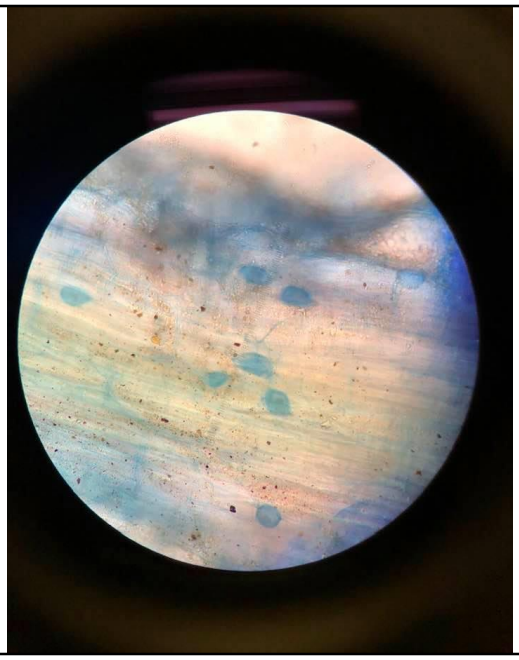


- To prepare the roots for observation: I followed the method first described by Phillips and Hayman, which my research partner (Janine) and I further tweaked and modified.
- I first attached the three segments to a labelled binder clip, cleared the roots in 10% potassium hydroxide, then acidified and stained using a 0.05% Trypan Blue and lactic acid solution, destained in a 1:1 ratio of lactic acid and glycerol, and finally cut each root segment to 1 cm long and mounted in glycerol

METHODS – Quantifying root colonization



- The quantification gets a bit tricky… but to ensure consistency in the volume of root being observed for each sample, a Field of View method was used…
- Each 1 cm long segment viewed with the 10x objective lens generated five FOV's (the five blue circles in the top/right). I omitted the outer two FOVs due to a high degree of root damage and poor visibility through the microscope.
- For each FOV I counted the number of vesicles and calculated the corresponding volume. Values are therefor represented as the # of vesicles per millimeter cubed.
- Nine measurements were used (three FOVs per three segments) to generate an average value for each plant.



This is an example of a FOV where vesicles are present.

METHODS – Experimental design

VARIETY	LOW PH	HIGH PH
Katani (dioecious)	**10**	**10**
X59 (dioecious)	**7**	**7**
Ferimon (monoecious)	**7**	**8**

- The experimental design had six treatments (three varieties of hemp (two of which are dioecious and one monoecious) and two different soils) with sample sizes ranging from 7-10 for each treatment

METHODS – No Difference in soil pH

- Main variable irrelevant
- Other potential differences between soil types
- “Soil A” and “Soil B”

- Now as I’m sure many people know, in the realm of research, things rarely go as planned…
- Near the end of my research, I decided to test the pH of the soils to generate more accurate values instead of simply referring to them as “high” or “low”. I discovered that the soils, in fact, didn’t display any difference in pH, and in some cases, the supposed “low” treatment was actually higher than the “high” treatment it was being compared to
- There are several possible explanations for why this occurred, but regardless of the reason, this discovery changed the direction and original focus of my study at the last minute.
- However, beyond pH, there could still be meaningful differences between the two soil locations, so I still carried out statistical analyses using my original data, but instead of using pH as a variable, I simply have soil A and soil B

STATISTICAL ANALYSIS – Two-way ANOVA

- Used SPSS software
- Failed to meet assumptions

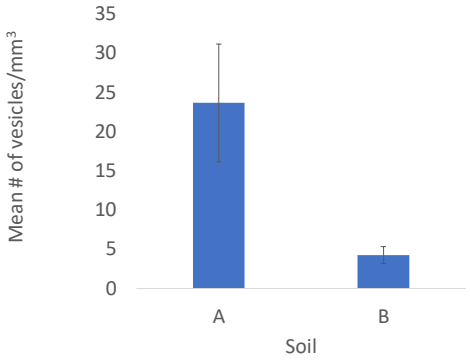
TREATMENT	P-VALUE
Katani – A	0.061
Katani – B	0.492
X59 – A	0.018
X59 – B	0.000
Ferimon – A	0.010
Ferimon - B	0.000

- Using a two-way ANOVA I first tested for any interactions between the two variables and colonization
- Unfortunately, most of the treatments Violated the assumptions of normality and equal variances as per the Shapiro-Wilk test and a Normal Q-Q plot. Even after applying several transformations, I was unable to do parametric tests.
- the katani treatments, however, were the only two to have normal data.

RESULTS – Two sample t-test

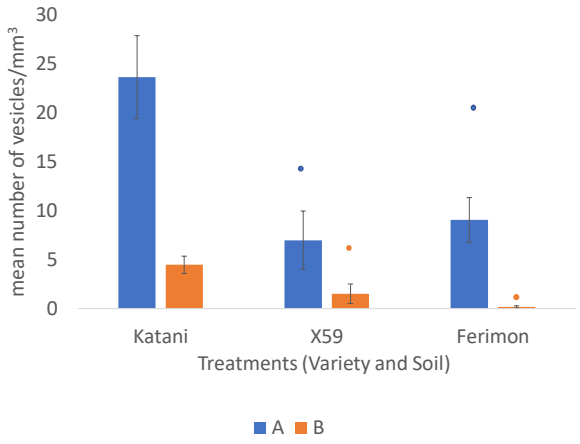
- Used only the Katani variety
- Significant difference

(df = 18, P = 0.020)



- From this, I decided to only look at the katani treatments and conduct a simple two sample t-test
- Here we can see a significant difference in colonization between the two soil types.

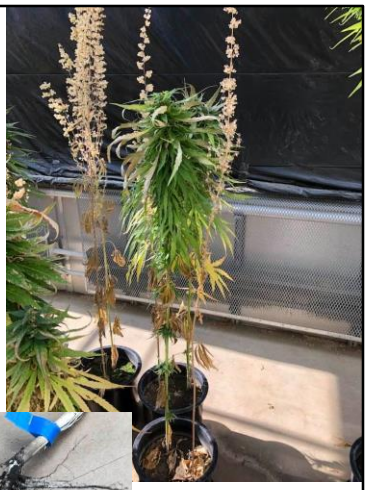
RESULTS – General trends

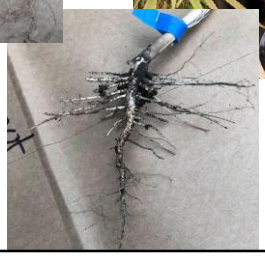


- I also conducted descriptive stats To identify any general trends in the data.
- This bar chart represents the averages of each treatment. The standard error bars suggest differences between the two soils in all varieties. However, the outliers in X59 and Ferimon varieties greatly skew the data, and make it difficult to draw any conclusions.

DISCUSSION - Limitations

- Small sample sizes
- Small root samples
- Males vs. Females
- Unable to use pH
- Confounding variable





- There are obvious limitations to my study. Firstly the samples sizes are quite small, ranging only from 7-10 depending on treatment
- Also, The root segments I cut were quite small, and may not have accurately represented the entire root system
- With Two of the hemp varieties being dioicous, the males matured much earlier than the females and monoecious variety.
- Of course there was the issue with pH
- And lastly, InnoTech's original experiment where I took plant and soil samples from, looked at the effect that different levels of cadmium have on plant growth, so it's possible that the amount of heavy metals in soil has a stronger effect on mycorhhizal colonization

DISCUSSION – Data still useful

- More meaningful soil variables
- Parasitism/mutualism debate

https://images.app.goo.gl/dNHrc2aPct71tB7U8

- Regardless of the limitations, the data I collected throughout this study is still useful and valuable to the scientific community.
- A more in-depth soil analysis may expose more meaningful variables regarding the soil types such as nutrient content, or the presence of fungicides and other toxins. It would also be useful to incorporate data of which crops were grown in these two areas in previous years.
- Additionally, by comparing my colonization data with Innotech's data on seed yield, plant height etc., we could identify trends between mycorrhizae and the health of hemp plants. This would provide valuable information towards the parasitism/mutualism debate, and determining the direction of this symbiosis.
- Once this relationship is better understood, we can further improve hemp agronomy practices, as well as set straight potential misinformation regarding the relationship between mycorrhizae and Cannabis.

To conclude my presentation, I would like to thank: my research supervisor Dr. Karen Christensen-Dalsgaard (for all of her guidance and expertise), my research partner Janine van der linden (for her moral support both in and out of the lab), and InnoTech Alberta for allowing me access to their experimental plants.

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