

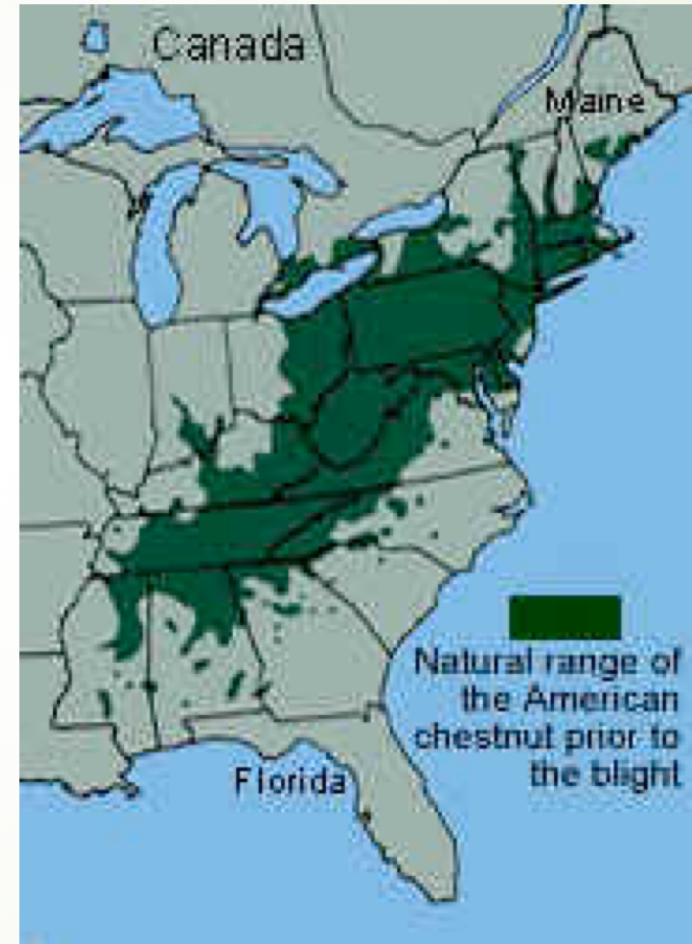


Microbiomes – how important are they?

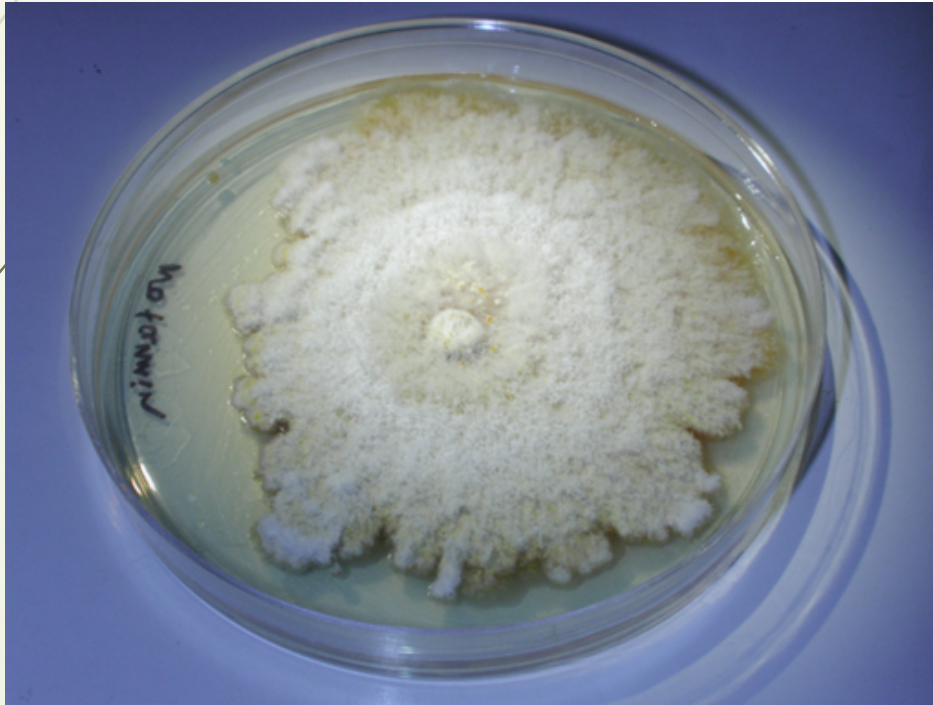
Dr. Laurel Rodgers

Shenandoah University

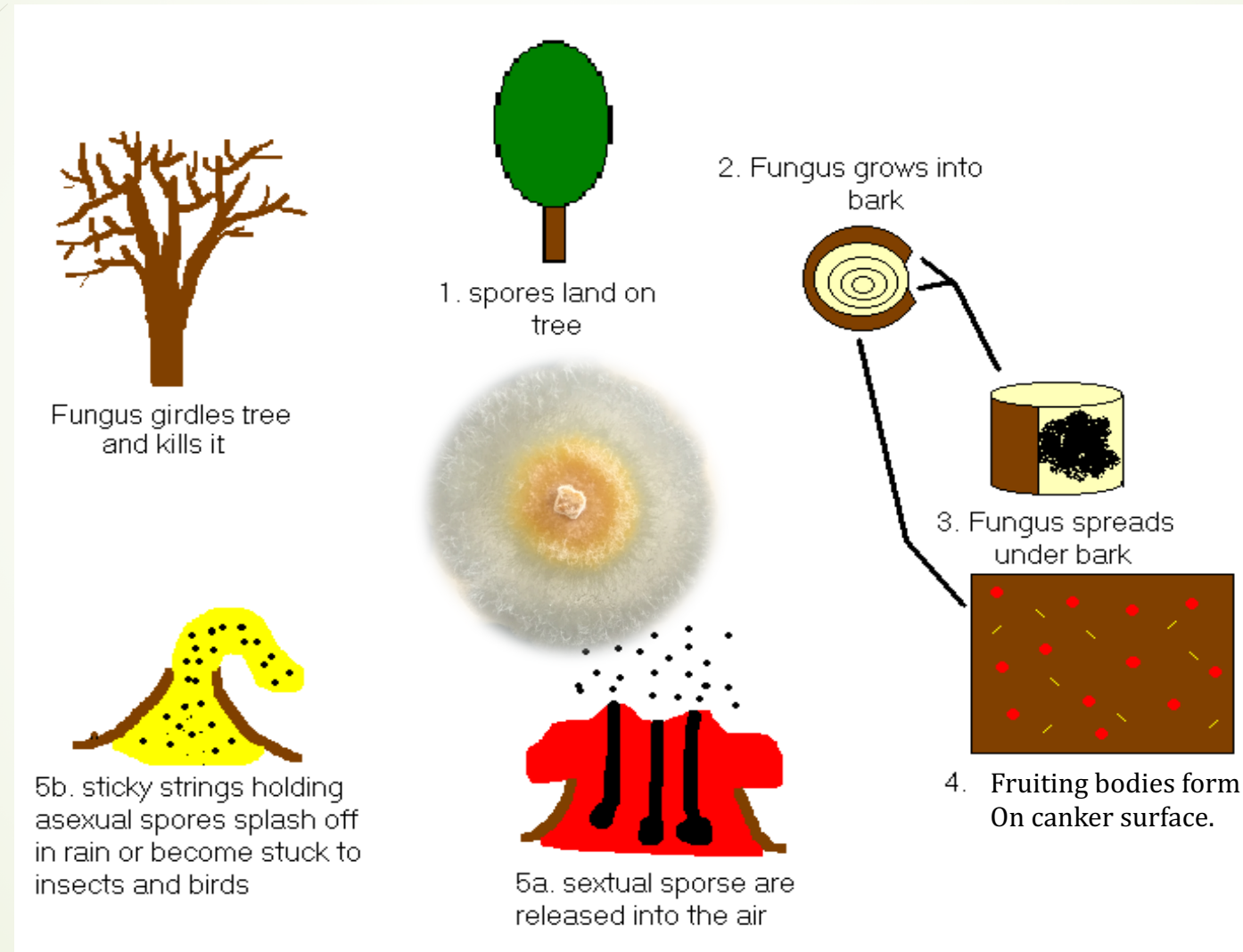
The American Chestnut



Cryphonectria parasitica and chestnut blight



C. parasitica life cycle




The American chestnut today

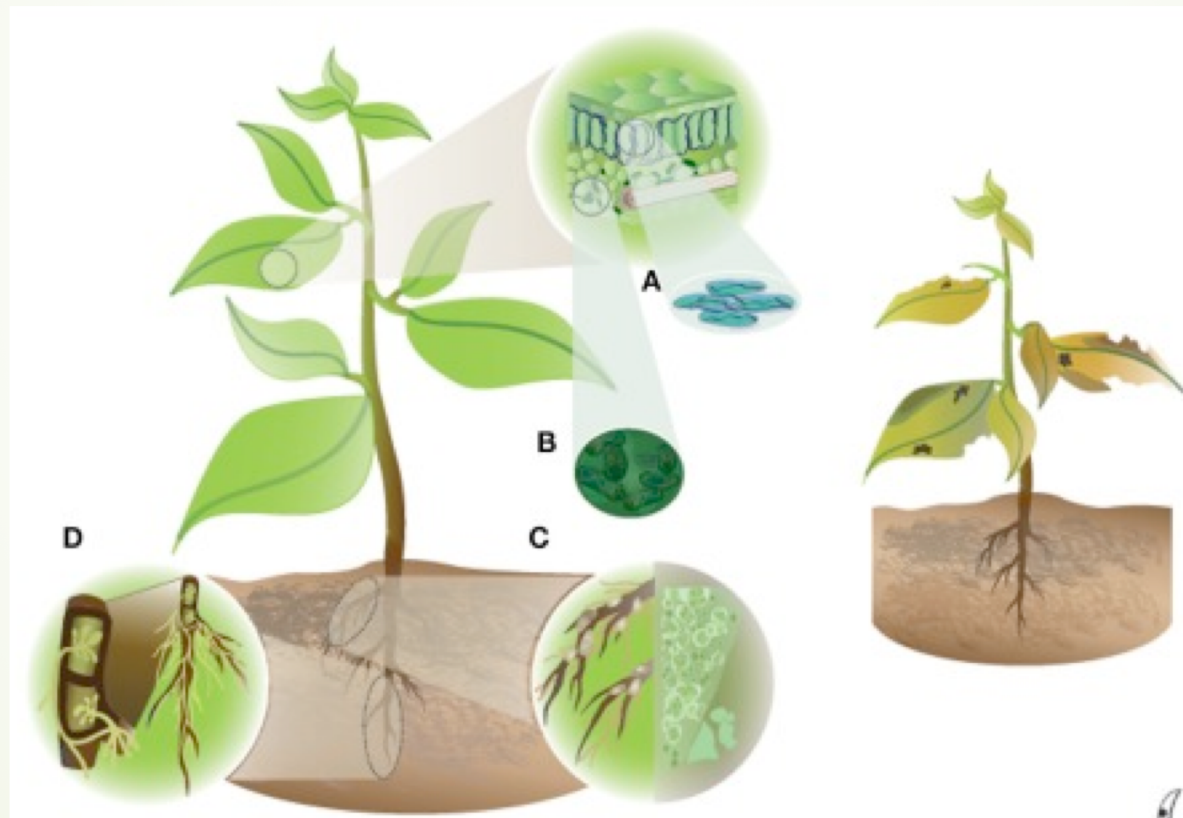




Restoration of the American chestnut

- Hybrid Trees
 - Genetic Engineering
 - Hypovirulence
 - Endophytes?
- 

Endophytes – Plants have a microbiome too!



Tree Fungal Diversity

Norwegian Spruce¹: 84 species on needle surface



48 species in tree



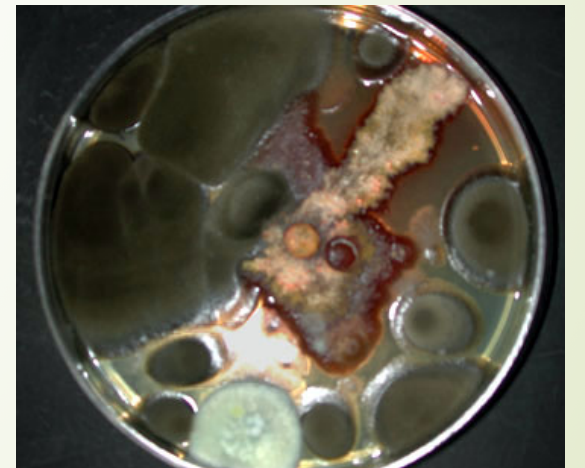
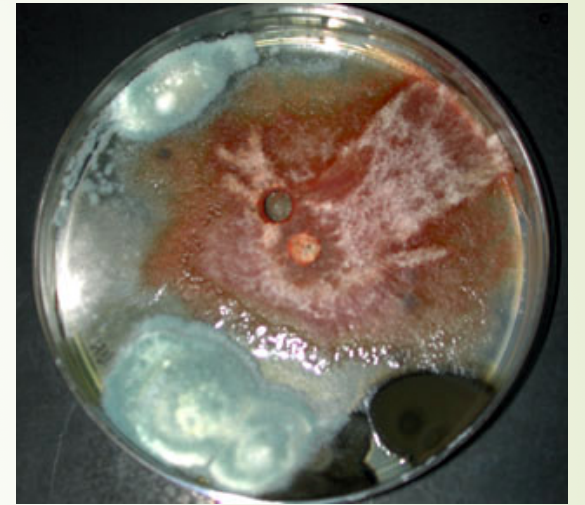
Balsam Fir²: 771 fungal isolates in sterilized needles


English Oak³: 60 species under dead bark
14 species under live bark



Endophytes as a biological control?


- Produce antimicrobial chemicals
- Increase plant pathogen response in plants
- Out compete harmful microorganisms (Danti et al. 2002)
- Reduces leaf damage and loss due to harmful pathogens (Arnold et al 2003)
- Produce toxins against pathogens
- Produce beneficial growth substances
- Inhibit growth of harmful pathogens by outcompeting them (Danti et al. 2002)






The Question: Are the endophyte populations in American and Chinese chestnut trees different?

What about trees with different levels of resistance?

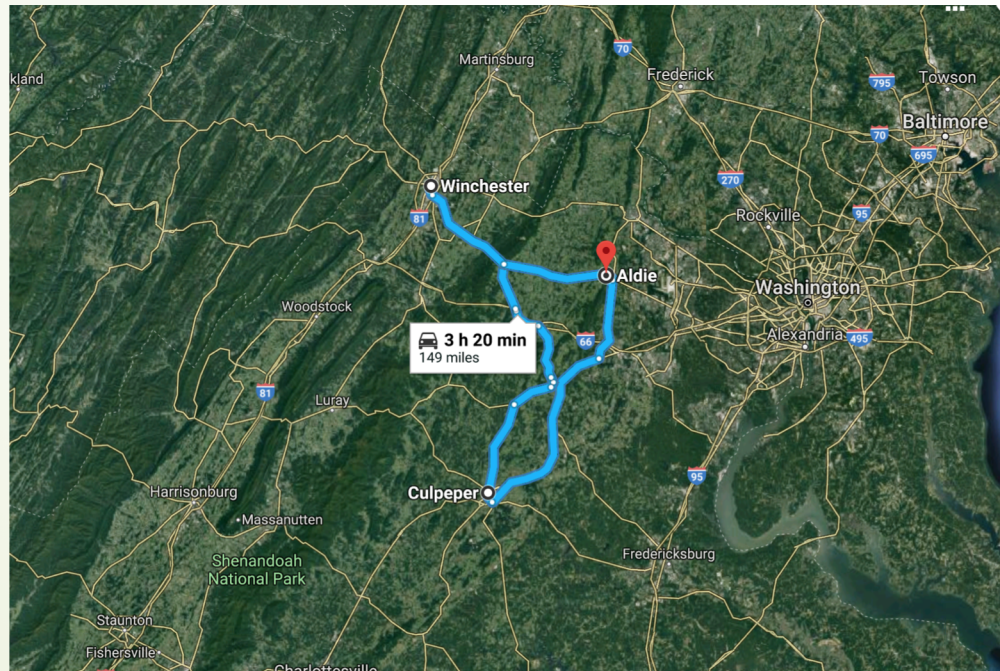


The Study: Compare fungal microbiomes of Chinese, American, and hybrid chestnut trees growing in the same location.



Collection sites

- Two orchards (June 2018)
 - The Ranch (Culpepper, VA)
 - Mount Zion (Aldie, VA)



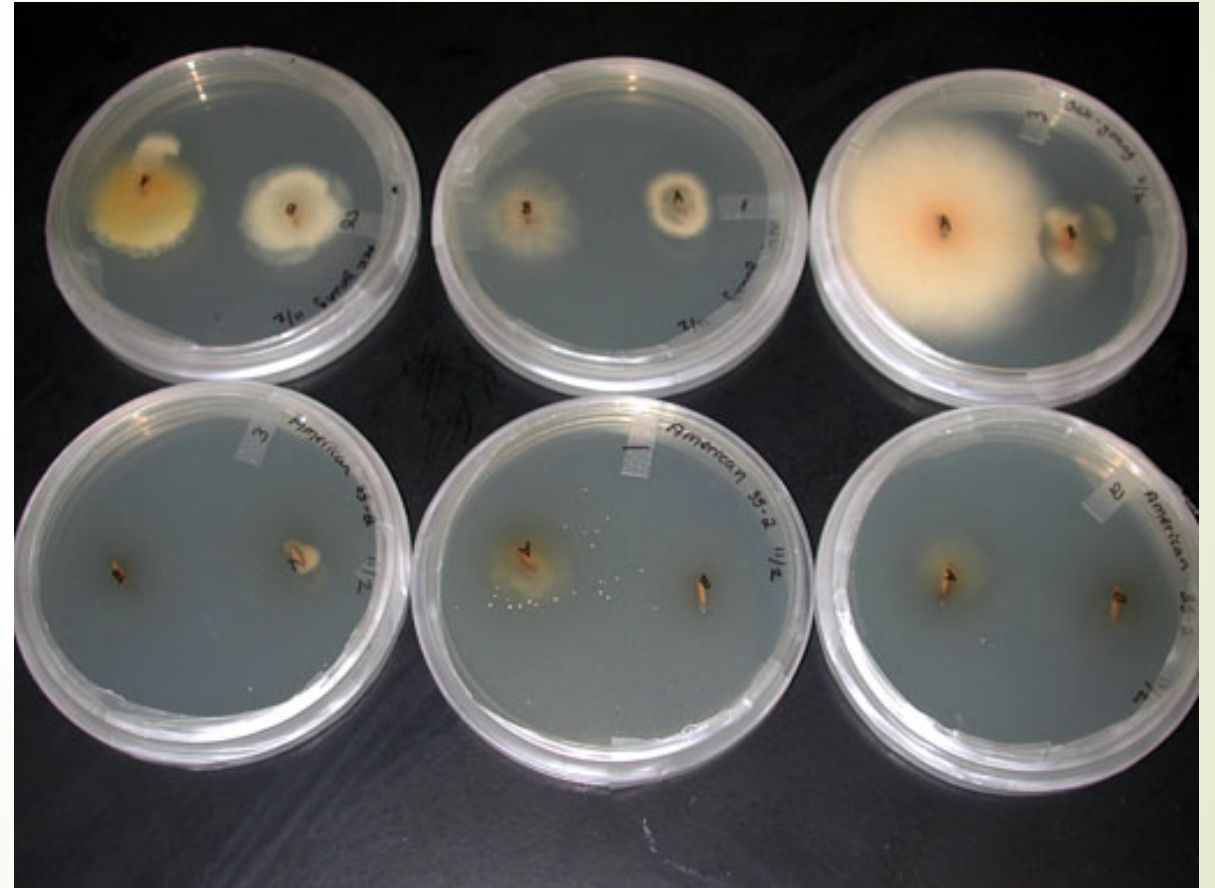
Bark Plug Collection

- Surface sterilize healthy bark
- Remove bark plug – take back to lab
- Inoculate tree with *C. parasitica*

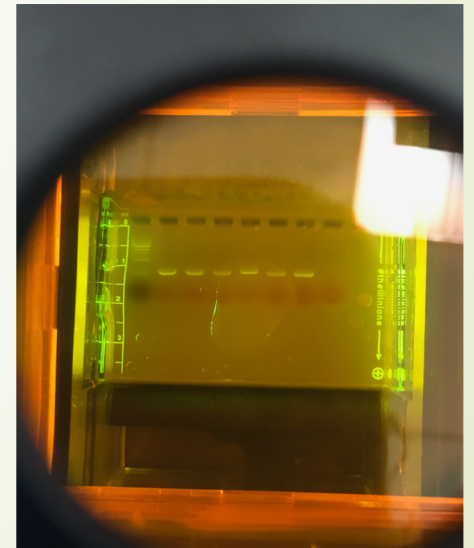
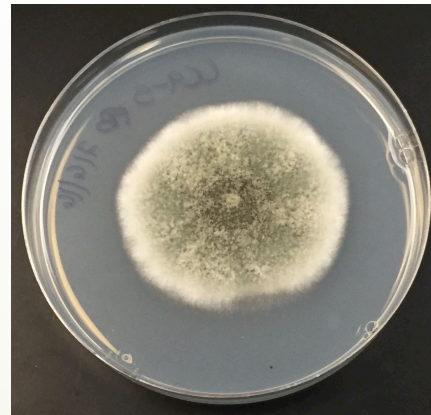
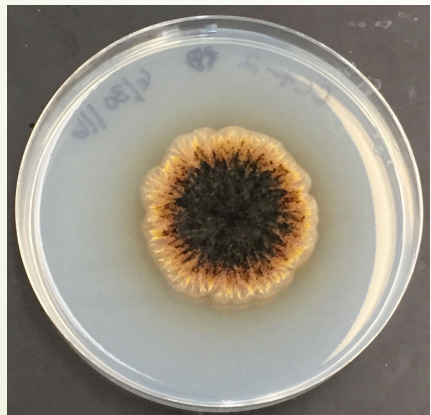
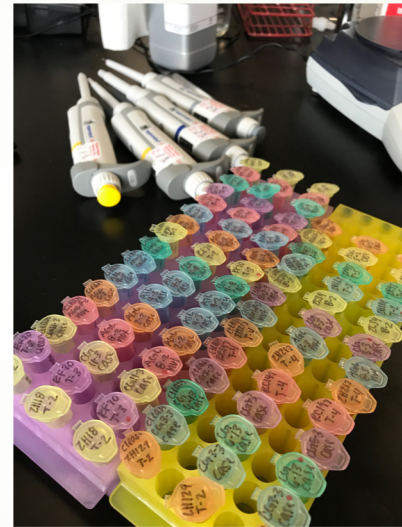
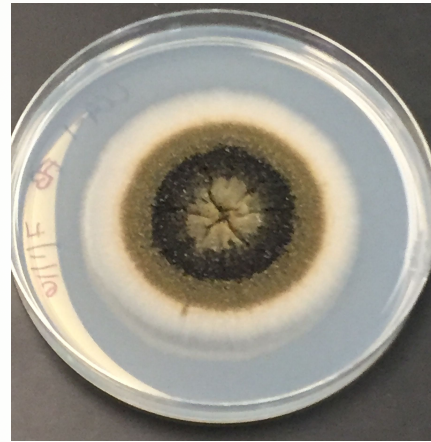
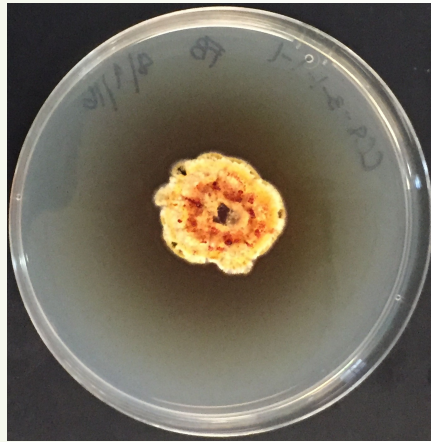


Collect Fungi samples

- Place bark in water agar
- Remove hyphae as they grow from bark
- Culture hyphae in nutrient agar

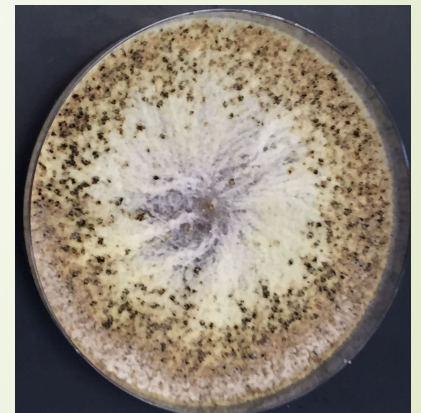
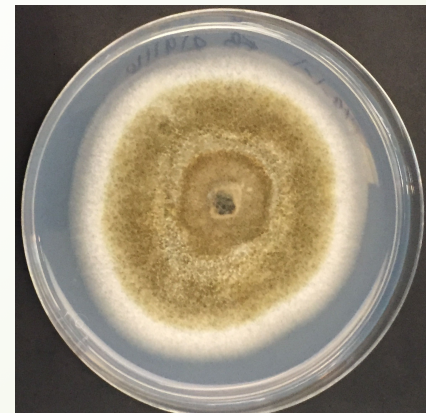



Grow Isolated Fungi and use DNA for Sequencing



What we know so far (it is a work in progress)


- There is some overlap in fungal species in each tree species.
- There are a lot of differences between trees
- We have found some fungi species that are considered endophytes in other plants
 - *Fimetariella rabenhorstii*
 - *Hypoxylon submonticulosum*
 - *Albifimbria verrucaria*





What's next?

- ▶ Finish identifying isolated fungi
 - ▶ Complete microbiome comparison

 - ▶ Are there endophytic fungi within chestnut trees?
 - ▶ How do we use endophytes to increase resistance?
- 



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