Microbiomes – how important are they?

Dr. Laurel Rodgers
Shenandoah University
The American Chestnut

https://www.acf.org/md/chestnut-background/story/
Cryphonectria parasitica and chestnut blight
C. parasitica life cycle

1. Spores land on tree
2. Fungus grows into bark
3. Fungus spreads under bark
4. Fruiting bodies form on canker surface.
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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