Santiago Rivera Reyes | Project proposal

**Evaluating growth patterns of Beech Bark Disease fungal agents *Neonectria faginata* and *Neonectria ditissima* under different *in vitro* conditions.**

*Background and rationale*

American beech (*Fagus grandifolia*) is a foundation species and a major component of eastern North American forests (Cale et al., 2017). Beech impacts soil health by preventing acidification and by helping to maintain a balance of nutrients (carbon, nitrogen and phosphorus) and soil organic matter (SOM) all favorable for tree growth (Ellison et al., 2005). These factors also favor a diverse soil microbiome and nutrient cycling which is beneficial for the whole forest ecosystem (Uroz et al., 2016).

Ever since its introduction in 1890 in eastern Canada, beech populations throughout infested regions have experienced heavy aboveground mortality from beech bark disease (BBD), a decline disease consisting of both insect (*Cryptococcus fagisuga* [the felted beech scale]) and fungal (*Neonectria faginata* and *Neonectria ditissima*) components (Ehrlich, 1934; Houston, 1994). The disease progression caused by this complex has been shown to have discrete stages: advancing front, killing front and aftermath forest (Cale et al., 2017). Further, the prevalence of the fungal species has been connected to the stage of disease, and the different species, in turn, are associated with different stages of tree decline (Morrison et al, 2021). Given broad geographic distribution and effects on forest stand structure and diversity, BBD is currently among the most important forest diseases in eastern North America (Rumble et al, 2020).

Studies on the fungal components of BBD have shown that *N. faginata* and *N. ditissima* co-occur throughout the range of BBD in the northeastern United States and how co-occurrence is associated with disease progression, tree decline and regional climate (Stauder et al., 2020, Morrison et al. 2021). Nevertheless, currently we lack an understanding of what regulates the prevalence of the fungal disease agents as well as how they respond to environmental variation.

There are several factors that have been established as important for the disease progression in the field. First, temperature and climate have a strong impact on BBD since the scale insect initiating agent appears to be limited by winter cold (Kasson & Livingston, 2012), and early and late season precipitation in part predict scale insect and fungal population dynamics (Garnas et al. 2011b). Some researchers have also found that *Neonectria* infections can be positively associated with dry conditions (Wiggins et al., 2004; Griffin et al., 2003). It has also been suggested that bark nitrogen levels can influence disease severity (Cale et al, 2017) and that low soil and bark phosphorus have a significant effect on infection by both *N. faginata* and *N. ditissima* (Cale et al., 2015). Water availability, on the other hand, has been studied as an influencing factor on the outcome of fungal competition for some species of fungi (Hurley et al, 2012). Finally, silicon (Si) concentrations have been shown to correlate with tree resistance to biotic and abiotic stress in some systems (Ahhammed & Yang, 2021) including beech bark disease (Nat Cleavitt, *personal communication*). Nonetheless, none of these factors have been studied in depth under controlled laboratory conditions.

My overall objective is to examine growth rates, spore production, and competitive hierarchies in the two fungal disease agents of BBD under controlled laboratory conditions, while systematically manipulating factors of potential ecological relevance for this disease in the field. Factors to be manipulated include temperature, nitrogen, phosphorous, silica concentrations, and water potential.

*Summer 2023, Research Objectives and approach*

I will conduct replicated growth and competition assays with a total of 18 previously collected isolates: 10 *N. faginata* and 8 *N. ditissima* from across five states including ME, NH, PA, WV and MI. These isolates where collected as part of an earlier study (Morrison et al, 2021) and are the subject of ongoing genomic work. First, I will evaluate all 18 isolates individually under different concentrations of N, P and Si and different temperatures following a full factorial experimental design. Precise concentrations and temperatures to be tested will be based on a combination of literature, pilot experiments and field-based measurements in trees. With this information, I then plan to pair up the different strains from the same state (*N. faginata* vs *N. ditissima*) and measure their growth under the concentration(s) that had the highest growth rate from the previous experiment. Additionally, they will be cultured using distinct water potentials as a variable (Hurley et al, 2012).

*Methodology*

The effects of nitrogen, phosphorus and silica on fungal growth will be evaluated using standard techniques (Balouiri et al., 2016). Briefly, a 0.5 cm diameter disc of fungus will be placed face down at the center of a 10 cm diameter plate containing malt yeast agar (MYA) amended with a low and a high concentration of the nutrients. All isolates will then be incubated at two different temperatures. The long and short diameters of the growing fungal culture will be measured using a digital caliper every second day from the fourth day after inoculation, for a total of 18 days.

The competition assays will be performed following established techniques (Klepzig & Wilkens, 1997). A 0.5 cm diameter disc of mycelium (fungal mat) from each *Neonectria* species (*N.ditissima* and *N.faginata*) from the same state will be placed on opposite sides of a 10 cm diameter plate containing MYA amended with the concentration(s) of nutrients that had the highest growth in the previous experiment. Growth rates and proportion of the plate occupied will be measured every other day for a total of 9 weeks using a digital caliper, and evidence of overgrowth or the formation of chemically-regulated barrier zones will be noted. Each assay pair will be performed in triplicate across the same range of experimental treatments and factor levels.

*Contributions to the field*

I will systematically select and pair fungal isolates of *N.faginata* and *N.ditissima* collected from across the range of BBD. The geographic scope of these collections will shed light on potential genotypic and geographic variation in fungal phenotypic responses to environmental variation. This study will provide valuable information regarding key performance parameters of these two fungi across a range of ecological conditions of hypothesized importance to disease etiology and epidemiology. This work will be enhanced by genomic work currently underway in the Garnas lab, creating many opportunities for synergy. Furthermore, the competition assays will contribute to the understanding of the disease dynamics, since it will provide deeper insight in the co- occurrence of both *N. faginata* and *N. ditissima*. Finally, the different variables involved in the experiments will provide knowledge about possible tree resistance mechanisms that can potentially be applied to management practices in the field.

*Literature cited*

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