1. Introduction

American beech (*Fagus grandifolia*) is a foundational species and a major component of eastern North American forests (Garnas, Ayres, et al., 2011b; Houston, 1994). As a nut producing tree, American beech is an important food source for forty wildlife species including black bears, deer, birds, and small mammals (Reed et al., 2022). Beech also 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).

Since the introduction of beech bark disease (BBD) in 1890, beech populations throughout different regions in North America 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: the advancing front, the 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).

*Neonectria faginata* and *Neonectria ditissima* (Ascomycota: Hypocreales, Nectriaceae) are characterized by the production of red, globose perithecia within which hyaline ascospores are found. They also have an asexual state (Mantiri et al., 2001), producing micro and macro conidia in sporodochia, both structures are responsible for the infection of beech trees, however asexual spores burst through the bark before the perithecia appear, often in the fall (Houston, 1983).

Despite similar morphology and disease etiology on beech, there are a few features that make *N. faginata* and *N. ditissima* different. Ascospore length which ranges from 12 µm for *N. faginata* to 16 µm for *N. ditissima* (Stauder et al., 2020). Nonetheless, perhaps the most differential aspect between the two organisms is that *N. ditissima* is a broad generalists, which infects over 130 host plant species belonging to at least 22 different plant families in both Europe and North America (Castlebury et al., 2006; Kasson & Livingston, 2009; Walter et al., 2015). Some of these non-beech host include: mountain maple (*Acer spicatum* L.), black birch (*Betula lenta* L.), mountain ash (*Sorbus americana*) (Stauder et al., 2020), apple trees (*Malus* sp.) (Gelain et al., 2020), white birch (*Betula papyrfera*), yellow birch (*Betula alleghaniensis*), eurpean alder (*Alnus glutinosa*), Red alder (*Alnus rubra*) (Kasson & Livingston, 2009, and references contained therein), white oak (*Quercus rubra*), chestnut oak (*Quercus alba*), American chestnut (*Castanea dentata*), american elm (*Ulmus americana*), eastern white pine (*Pinus strobus*), black spruce (*Picea mariana*) (Castlebury et al., 2006; Ghasemkhani et al., 2016; Gómez-Cortecero et al., 2016) to name a few. *Neonectria faginata* on the other hand, has only been observed causing annual cankers on American beech trees following beech scale infestation (Castlebury et al., 2006).

An unanswered question in the beech bark disease system is how *N. ditissima* and *N. faginata* coexist. Niche theory predicts that when two species are occupying the same niche, one of them will outcompete the other essentially causing the exclusion of the less competitive species out of the system, therefore it is not clear how *N. faginata* and *N. ditissima* continue to coexist within the same niche.

Many researchers have in fact described a pattern of competitive exclusion whereby *N. ditissima* arrives first after the scale insect and is replaced by *N. faginata*. For instance, long-distance dispersal events associated with the felted scale has resulted in a gradient of duration of infestation depending on the time of the arrival of the scale insect to the trees and researchers have found that *N. ditissima* is more prevalent in the killing front of the disease (which is defined as tree stands where the insect arrived from 10 to 20 years) whereas *N. faginata* dominates aftermath forest, which suggests that it replaces *N. ditissima* across the range of BBD after 7 years of apparent tree infection (Houston, 1994; Morrison et al., 2021).

Another explanation that derives from niche theory is the host range tradeoff. As mentioned, *N. ditissima* is a generalist pathogen and it has been suggested that the reason why it is always found on *N. faginata* infected beech trees is because other hardwood susceptible tree species with numerous stem or branch cankers produced high inoculum loads, and therefore the diversity and abundance of these alternative hosts in beech stands allow for the colonization and establishment of *N. ditissima* (Kasson & Livingston, 2009).

Additionally, niche differentiation could also be at play, as it has been reported that although *N. faginata* can completely replace *N. ditissima* in most forest stands, both fungi are shown to be present in more recently infected trees (Kasson & Livingston, 2009), which suggest that they may specialize in different areas of the tree which allows for co-existence between them.

However, trade-offs between these two species seem to be the most plausible explanation based on the evidence. One example of trade-off is how each species behave in relationship to climate and temperature. Morrison et al (2021) found that heat accumulation was related to incidence of both species, however they concluded that both species have divergent climate associations whereby *N. ditissima* was prevalent in colder climates while *N. faginata* was associated with warmer climates. They also found that heat accumulation was the strongest predictor of *N. faginata* occurrence while *N. ditissima* incidence was positively associated with freeze-thaw cycle frequency. Nonetheless, it is still unclear how temperature directly affects the growth and the ecological dynamics of these two species.

One of the most studied predisposing factors that contribute to the infestation by *N. faginata* and *N. ditissima* has been nutrition. As previously mentioned, nitrogen influences fungal behavior and ecological dynamics. Specifically, in the case of the infection by *N. faginata* and *N. ditissima* high bark N levels are related to a higher severity of the disease (Latty et al., 2003).

Nitrogen is not the sole nutrient involved in the dynamics of BBD. Phosphorus has also been shown to play a key role in the progression of the disease For instance, Cale and collaborators (2015) identified positive association between low bark P and *N. ditissima* infection when combined with other factors. High foliar N:P ratio was also associated with a higher probability of infection by both fungi (Cale et al., 2017). This suggests that variation in nitrogen and phosphorus in the soil or in bark could favor context-specific conditions that might favor one species over the other locally. Alternatively, one fungus may thrive under certain nutrient environments while the other takes over when the concentration of nitrogen or phosphorus change. Regardless of the interplay between nitrogen and phosphorus concentrations, the nutrient interaction with temperature and how that translates into the growth and/or aggressiveness of *Neonectria* spp. fungi remains unclear.

The objective of this study was to examine how different levels of nitrogen, phosphorus and temperature influence growth, and competitive dominance between *N. faginata* and *N. ditissima* through the use of established microbiological techniques under controlled laboratory conditions. This study aimed to elucidate how nutrients and temperature can potentially contribute to subtle differences in niche requirements of these two species which might provide a potential explanation for context-specific growth, survival and ultimately coexistence of *N. ditissima* and *N. faginata* on infected beech trees.

We hypothesized that the growth of *N. faginata* and *N. ditissima* is influenced by the variation in nitrogen (N), phosphorus (P) and temperature in distinct ways, driven by a trade-off mechanism wherein each species thrives under specific conditions. These differences may lead to different outcomes in their interactions with the host tree. Faster growth by the fungi could increase the likelihood of resource acquisition for reproduction, potentially outpacing the tree’s defensive response.

Secondly, we hypothesized that variation in N, P and temperature can influence competitive interactions between *N. faginata* and *N. ditissima.*  We aimed to investigate whether there is evidence of a trade-off in the use of nitrogen or phosphorus across different temperature conditions, which could determine the dominance of one species over the other based on the nutrient and temperature environment. Furthermore, we explored how these nutrients and temperature shape the nature of competition, particularly whether the hyphal behavior of *N. ditissima* or *N. faginata* depends on encountering its congeners and how it changes in different nutritional contexts.