Graduate Research Plan Statement

Introduction: In recent decades, reports of re-emerging and novel phytopathogens have increased dramatically in forests.[1] These pathogens threaten forest health and pose serious risks to plant biodiversity. Studies indicate climate change (e.g. warmer temperatures, wetter growing seasons) has accelerated forest decline within the United States by expanding plant pathogen ranges.[2] **The effects of climate change have heightened and extended the infection period for pathogens, making trees more vulnerable to outbreaks of less aggressive phytopathogens.**[3] Plant pathogens in the family Nectriaceae, including undescribed species, have been indirectly linked to climate change.[4] In addition, these changes in temperature are known to increase sporulation and virulence of fungal pathogens, as cold periods would ordinarily reduce the populations of pathogens by arresting their growth. Trees at high elevations including red spruce, Fraser magnolia, yellow birch, striped maple and mountain ash are buffered from many pathogenic fungi due to persistent cold temperatures in their habitat; however, warmer winters have increased the risk for biological invasion of these species.[5]

Although significant progress has been made regarding the taxonomy of these nectriaceous fungi, additional data are needed to clarify species boundaries and their evolutionary relationships. Likewise, these fungi pose risks that must be fully assessed by more robust studies on host range and pathogenicity. Long studied forests are now experiencing epidemics of these emergent plant pathogens (EPPs).[6] While beech bark disease and *Fusarium*-associated diseases are highly-studied pathosystems, native, often less virulent, nectriaceous fungi are becoming more abundant. My objective is to protect Appalachian forests by 1) drawing connections between abiotic stressors and the prevalence of nectriaceous fungal pathogens, 2) identifying these fungal pathogens, and 3) assessing the effects of temperature on the aggressiveness of these pathogens. **I propose to expound upon current climate change models and forest pest predictions, particularly for nectriaceous fungi on these high-elevation tree hosts.**

Aim 1: Assessing abiotic stressors contributing to the emergence of fungal pathogens.
Using the 'Climate by Forest' tool provided by the U.S. Forest Service, I will review changes in forest health and climate projections for forests throughout the Appalachian region. The 'Climate by Forest' tool is a novel interface in which users can select regions of national forests and look at various climate trends and variables.[7] From these projections, forests that are predicted to have significantly warmer winters will be selected for sampling. High-elevation tree species will be selected based on their known range across high elevation zones throughout Appalachia. Symptomatic tissues and conspicuous fungal fruiting bodies from these species in our sample sites will be surveyed, collected and processed for culture- and DNA-based studies. I will also compile and analyze temperature data across the Appalachian region to quantify and evaluate the abiotic stress these forests have endured. **Hypothesis: Fungal pathogens and abiotic stresses are synergizing declines in native tree species.**

Aim 2: Characterizing known and unknown nectriaceous fungal diversity in Appalachian forests.
Hypothesis: Despite known diversity of Nectriaceous fungal pathogens across Appalachian forests, many remain undetected. Molecular tools must be used in combination with existing morphological methods to capture the full diversity of phytopathogenic fungi. Sanger sequencing will be used for pure cultures of my suspected fungal pathogens recovered from trees sampled in *Aim 1*. Targeted loci (LSU and EF1-α) are widely used for phylogenetic inference in Nectriaceae.[8] Illumina amplicon sequencing—a multiplexed PCR approach—will be used to identify asymptomatic fungi that may also be contributing to forest decline.

Aim 3: Determining the interaction between individual nectriaceous fungi and targeted tree species in central Appalachia along a temperature gradient. **Hypothesis: Nectriaceous fungi have contributed**

differentially to tree disease epidemics, which are driven in part by changes in temperature throughout our Appalachian forests. To simulate global warming and to assess the effects of temperature on fungal growth and pathogenicity, temperature-dependent pathogenicity assays will be conducted. In climate-controlled growth chambers, saplings of the aforementioned five species will be grown at varying temperature ranges (0°C, 10°C, 20°C, and 30°C). Trees will be inoculated with select nectriaceous fungi discovered in *Aim 2* and tree health will be monitored at 6-MPI and 12-MPI. Any notable canker formation will be measured at the end of the inoculation period. To fulfill Koch's postulates, trees will also be sampled to see if the original inoculum can be recovered. This experiment will quantify the aggressiveness of suspected nectriaceous pathogens at varying temperatures, allowing me to infer the impact novel phytopathogens will have on our forests as global warming worsens.

Intellectual Merit: As a member of Dr. Kasson's forest pathology lab since mid-2020, I have direct experience identifying and characterizing diverse fungal phytopathogens in West Virginia. During my efforts to delimit the species boundaries of *Neonectria magnoliae*, **I have already identified numerous nectriaceous fungi on a wide range of hosts, including some novel species we are describing**. Putative pathogens in the Nectriaceae are abundant and appear to be emerging as the result of the unique overlap of biotic and abiotic factors. I have and will continue to collaborate with forest pathologists throughout the Appalachian region to compare DNA sequences, host range, and pathogenicity of these fungi under supervision of Dr. Matt Kasson (WVU), my current advisor and forest pathology expert. With his support and the support of my forest pathology colleagues, I will not only unravel the contributions of these fungal phytopathogens to the decline of our forests, but provide novel information to our Appalachian communities, our foresters, and our scientists. **West Virginia is the "black box" of biodiversity**: severely understudied with much to discover. There are an estimated 150 different tree species in WV: more than anywhere else in North America. My contributions to forest pathology will revolutionize how we look at and care for our trees in Appalachia.

Broader Impacts: West Virginia (WV), my home state, is suffering from educational neglect. It has the lowest number of Bachelor's degrees (20.6%) per capita of any state, and we have the second lowest per capita graduate degrees.[9] In Appalachia—and WV specifically—we deal with low science literacy and a fear of science. Our region once powered the country with coal, but the profits of this mono-cultural economy were not reinvested in our communities. As coal has faded away and global temperatures rise, there is much skepticism and fear around climate action in West Virginia. **West Virginians need scientists from our own communities trained to identify the challenges we face, develop solutions to these problems and share them with our own.** These scientists, like myself, will have a broad and immensely positive impact on my community.

As science outreach has been an integral part of my undergraduate career, I will curate my own environmental science outreach program to invest my project in our Appalachian youth. Through my **Appalachian Children's Environmental Research program** (ACER), I will recruit fellow young scientists to bring presentations to K-12 students and also offer field trips to teach a variety of environmental concepts. In this novel program, I will provide resources (i.e., guides, lessons, and accessible information) on climate change, forest health, mycology, and other topics pertaining to preserving the integrity of our ecosystems. My lifetime of learning has prepared me to fulfill this next stage of my career, one that will ensure West Virginians are not left behind.

References: [1] Karunarathna et al. 2021. *Front Cell Infect Microbiol*. [2] Kasson et al. 2009. *Mycologia*. [3] Dukes et al. 2008. *Can J For Res*. [4] Pavlov et al. 2020. *Sci Rep*. [5] Pauchard et al. 2015. *Biol Invasions*. [6] Corredor-Moreno et al. 2019. *New Phytologist*. [7] U.S. Forest Service. 2018. Climate by Forest. [8] Stauder et al. 2020. *Fungal Ecol*. [9] West Virginia QuickFacts. U.S. Census Bureau.