Are fungal endophytes of fire adapted plants also fire adapted?

Alex Dowd

Faculty Advisor: Dr. Karen Hughes

Chancellor's Honors Program Senior Thesis

Department of Ecology and Evolutionary Biology

University of Tennessee, Knoxville

Spring 2023

Abstract:

Fungal endophytes are fungi that asymptomatically inhabit living plant tissue. This study examines heat tolerance as a mechanism of endophytic succession at burn sites of the 2016 Chimney Tops 2 Fire in the Great Smoky Mountains National Park. We hypothesized that the majority of endophytes found at these burn sites would be heat tolerant and that a greater number of heat tolerant endophytes would be found in pine seedlings (fire-adapted, found in severe burn areas) than in *Rhododendron* shrubs (not fire-adapted, found in moderate burn areas). We cultured endophytes isolated from seedling needles and regenerating rhododendron leaves on malt extract agar plates. These isolates were heated at 65°C for treatments of 0, 10, 20, and 30 minutes. Their growth was monitored for two weeks, after which heat resistance was determined based on presence or absence of growth after 30 minutes of heating. Less than half of the endophytes we tested were heat resistant. Heat resistant endophytes were not significantly associated with fire adapted host plants. Intraspecific variation of heat resistance was found in many taxa, suggesting that endophytic heat resistance varies at the level of genotype rather than species. Possible mechanisms of genotype-level heat resistance include epigenetic effects, heat shock proteins, or thermostable cellulases. In the face of climate change, further research on fungal heat resistance could contribute to novel issues within the fields of fungal epidemiology and fire ecology.

Introduction:

Fungal endophytes are fungi that inhabit living plant tissues without negatively affecting the plant's health (Hirsch & Braun, 1992). Endophytes are present in all land plants (Stone et al., 2004), but the nature of their relationship with their host varies based on whether the host is a woody or herbaceous plant. Endophytes of herbaceous plants are usually vertically transmitted, meaning that the host plant passes their endophytes on to their offspring through their seeds. In contrast, woody plants' long lifespans make vertical transmission infeasible for their endophytes (Saikkonen et al., 2004). Thus, endophytes usually colonize woody plants through horizontal transmission, meaning that they spread spores aerially from plant to plant through passive dispersal (Wilson, 2000). Endophytes of woody plants are usually commensal (Alholm et al., 2002), but some are hypothesized to be mutualistic or pathogenic (Rodriguez et al., 2009).

In November and December 2016, a series of wildfires collectively referred to as the Chimney Tops 2 Fire burned through approximately 11,000 acres of the Great Smoky Mountains National Park (GSMNP) in Tennessee, USA (Hughes et al., 2020a; Miller et al., 2017). Previous research has focused on the ecology and taxonomy of successional pyrophilous fungi (fungi that grow and reproduce exclusively in post-fire habitats on burned or heated areas and are generally absent from fungal communities outside of fire systems (El-Abyad & Webster, 1968; Seaver, 1909)) found growing at Chimney Tops burn sites (Hughes

et al., 2020a; Hughes et al., 2020b; Matheny et al., 2018; Miller et al., 2017; Raudabaugh et al., 2020). When visiting the burn sites in 2017, researchers found fungal endophyte growths in the needles of successional pine seedlings at many of the sites (K. Hughes, personal communication, March 10, 2023). This unexpected finding raised a question: how did these endophytes arrive at the burn sites? The present study examines the possibility of heat tolerance as the mechanism of endophytes' survival of the wildfire and subsequent colonization of successional pine seedlings and *Rhododendron* plants at the burn sites. The purpose of this study was to compare the post-fire endophyte communities of fire adapted plants with those of non-fire adapted plants and to examine the heat resistance of endophyte species presence in each community.

Pinus is the sole genus of the conifer family Pinaceae. *Pinus* species are distributed across the northern hemisphere and are common throughout the eastern United States. Members of *Pinus* subgenus *Pinus* are fire-adapted, meaning that fire regimes are essential components of their life cycles. Many fire-adapted pines reproduce using serotinous cones, which contain seeds that are released when the heat of a fire melts the protective resin keeping them encased within (Keeley, 2012). After the Chimney Tops 2 fire in the GSMNP, seeds from table mountain pine (*P. pungens*) and pitch pine (*P. rigida*) dropped from serotinous cones and germinated, becoming primary successional seedlings. Some pitch pine seedlings developed from underground rhizomes that survived the fire. Pine served as our experimental host because of its fire adapted life cycle. A prevalence of heat tolerant endophytes in pine seedlings could indicate coevolution of endophyte life history traits corresponding to host plant life cycles.

Rhododendron is a large genus of woody plants in the family Ericaceae. The GSMNP is home to twelve species of *Rhododendron*, the most common of which is great laurel (*R. maximum*; GSMNP, 2022). Great laurel often grows abundantly in forests dominated by table mountain pine and pitch pine. Great laurel spreads quickly through open areas, often acting as a pioneer successor at burn sites. Though it has adapted to fire regimes in many locations, we did not consider *Rhododendron* as a fire adapted genus for the purpose of this study because fire is not an essential component of its life cycle (Anderson, 2008). For this reason, *Rhododendron* is the control endophytic host in this study.

Many species of non-endophytic fungi have been found to be heat resistant. Heat resistant, non-endophytic fungi often reside in soils, in which their spores may lie dormant until heating catalyzes their germination (Jesenska et al., 1993; Witfield et al., 2021). Saprotrophic fungi that are prevalent on burned or heated wood (also known as lignicolous fungi) have been found to survive temperatures between 140-220°C (Carlsson et al., 2012). Carlsson et al. (2012) suggest that heat shock proteins, which repair cells and protect proteins from denaturing, could be a mechanism of heat resistance in lignicolous fungi. It is possible that heat shock proteins could play a similar role in endophyte heat resistance.

A wide variety of researchers have investigated how endophytes affect the heat resistance of their host plants (e.g., Ali et al., 2018; Baynes et al., 2012; Hubbard et al., 2013;

Nagabhyru et al., 2022; Redman et al., 2002). Endophytes can confer heat resistance to their hosts by enhancing the expression of their hosts' heat shock proteins (Baynes et al., 2012) or by additively expressing their own stress-response genes in tandem with their host's stress-response pathways (Nagabhyru et al., 2022). The fact that endophytes are capable of bolstering heat tolerance in other organisms could be indicative of their own potential for heat resistance. One study raises the possibility that the filamentous fungus *Neurospora crassa*, which can be an endophyte of Scots pine (*Pinus sylvestris*), can survive wildfires from within the host plant's cells (Kuo et al., 2014). This has promising implications for the heat resistance of endophytic fungi.

The present study is, to our knowledge, the first to measure heat tolerance of foliar fungal endophytes in the contexts of post-fire succession and host fire adaptation. We hypothesized that most endophytes sampled from burn sites would be heat tolerant, meaning that they would survive heat shock and continue to grow afterward. We also predicted that a greater number of heat tolerant endophytes would be present in the needles of successional, fire adapted pine seedlings than in the leaves of successional *Rhododendron* shrubs.

Methods:

Collection and cultivation of endophytes:

In July 2017, following the Chimney Tops 2 fire, needles and leaves were collected randomly from 20 pine seedlings and coppicing shoots of *Rhododendron*, respectively. Pine needles were collected at a severe burn site on Baskin's Creek Trail and *Rhododendron* leaves were collected at an adjacent, moderate burn site on Baskin's Creek Trail. The collected leaves and needles were sterilized, washed with clean sterile water, and sectioned into 5mm pieces. These tissue fragments were surface-sterilized using sequential immersion in 95% alcohol (for 10 seconds), chlorine bleach (for 2 minutes), and 70% alcohol (for 2 minutes; Arnold & Lutzoni, 2007). The tissue fragments were cultured on malt extract agar (MEA; 15g Difco malt extract, 20g Difco agar) plates and observed every couple of days. Fungal growths were excised and isolated as they appeared. These samples were subcultured on MEA plates and left to grow until fungal growth covered the plates.

Taxonomic identification:

The fungal DNA barcode (ribosomal nuclear ITS region (nrITS); Schoch et al., 2012) was generated from the endophyte cultures by other workers following procedures outlined in Hughes et al. 2020a. Individual DNA sequences were compared to fungal ITS sequences in the National Center for Biotechnology Information (NCBI) using an imbedded search engine, the Basic Local Alignment Search Tool (BLAST), which compares the query sequence to all sequences in the NCBI database and produces the closest match to the query sequence. Endophyte sequences were provisionally assigned to the genus and species (if available) of

the best match. Where multiple sequences were assigned to the same genus, the endophyte nrITS sequences were compared with each other using the program Aliview (Larsson, 2014) to visualize the sequences.

Heat resistance:

65 endophytes isolated from the burn sites were tested for heat resistance. To test endophyte heat resistance, four sections of approximately 0.5 mm^2 were cut from each cultured isolate using sterile technique and placed individually on MEA plates. These plates were sealed with Parafilm and subjected to heat shock for 0, 10, 20, or 30 minutes at 65°C in a Fisher Scientific Isotemp incubator (Fig. 1). Following heat shock, the plates were left to grow in a climate-controlled room for two weeks. Fungal growth was measured four times during the growth period in centimeters of diameter growth from the culture sections. We operationalized heat resistance as the presence of growth from the excised endophyte sections after 30 minutes of heating. Isolates were deemed "heat resistant" if they had grown at all after 30 minutes of heating and "not heat resistant" if they did not grow after 30 minutes of heating.

We originally intended to test heat resistance at 220°C in a Blue M Single-wall Transite oven based on the heating regimes used by Carlsson et al. (2012) in their experiments on heat tolerance in lignicolous saprotrophic fungi. This temperature proved to be too hot for most equipment to withstand, and the oven did not reliably reach and maintain desired temperatures. We decided instead to heat specimens to 65°C, the maximum temperature of the Fisher Scientific Isotemp incubator in our lab. Dunn et al. (1985) found that 60-80°C was a maximum threshold for fungal survival of heat shock depending on soil moisture. Neary et al. (1999) proposed that microbial mortality occurs between 50-121°C of soil heating, with soil-dwelling fungi falling near the lower end of this range. In this experiment, 65°C of heating was the most feasible temperature for heat shock based on previous literature and equipment limitations.

For the first half of the experiment, endophyte isolates were grown in potato dextrose (PD) broth alongside the isolates grown on MEA plates. To test heat resistance in PD broth, we used the same methods of excising four sections of each endophyte isolate and heating them for variable durations at 65°C in the incubator. After being heated on MEA plates, we transferred each endophyte section to a 30mL jar of PD broth using sterile technique. Growth was measured by removing all fungal tissue from the jar, drying it in the Blue M Single-wall Transite oven until all moisture was removed, and weighing the dry mass of the growth in grams. Just as with the MEA plates, heat resistance was operationalized as the presence of growth after 30 minutes of heating. This method was ultimately discarded after the PD broth attracted ants that contaminated many PD isolates. Growth patterns in liquid media were also inconsistent with their MEA counterparts, making PD broth an unreliable medium for consistent measurement of heat resistance.

Data analysis:

Data were recorded, collated, and analyzed in Microsoft Excel (version 2302). A 2x2 one-tailed chi-square contingency test was used to determine if the difference in the proportion of heat resistant endophytes found in pine and *Rhododendron* hosts was significant. We wanted to analyze the diversities of endophyte communities in pine and *Rhododendron* in order to model post-fire endophyte diversity. Alpha diversity of pine and *Rhododendron* endophyte communities and beta diversity between the two host communities (Whittaker, 1972) were calculated in Excel. Rarefaction curves for pine and *Rhododendron* endophyte communities were calculated using EstimateS 9.1 and graphed in Excel.

Results:

Of the 65 endophyte isolates tested for heat tolerance, approximately 48% (n = 31) were heat tolerant. Heat resistance of isolates by taxon is shown in Table 1. Growth in response to heat shock varied among the endophyte isolates; some grew to cover their plates within days, while others barely grew at all after two weeks. Examples of variation in growth patterns are shown in Figures 1, 2, and 3.

Heat resistance varied intraspecifically for many of the species/genera with multiple representatives. For example, of the 14 specimens of *Sydowia polyspora* tested, 4 were heat resistant and 10 were not (Table 1). Figures 2 and 3 show the differences in heat resistance and growth pattern between two isolates of *S. polyspora*.

Rhododendron plants hosted significantly more heat tolerant endophytes than pine seedlings; 56.25% of *Rhododendron* endophytes were heat tolerant compared to 38.23% of heat tolerant pine endophytes (Fig. 4). *Rhododendron* hosted a greater diversity of endophyte species (alpha = 21) than pine (alpha = 18; Fig. 5), and there was little species overlap between the two endophyte communities (beta = 33; Fig. 6).

Table 1. Heat resistance of endophyte taxa isolated from burn sites.

Classification	Genus/species (*N*)	Host genus/species (*n*)	Heat resistant? (*n*(yes)/*n*(no))[a]
Ascomycota; Botryosphaeriales; Botryosphaeria	*Microdiplodia* sp. (2)	*Rhododendron* sp. (1)	0 / 1
		Rhododendron maximum (1)	0 / 1
Ascomycota; Cladosporiales; Cladosporiaceae	*Cladosporium anthropophilum*	*Rhododendron* sp. (4)	1 / 3
Ascomycota; Coniochaetales; Coniochaetaceae	*Coniochaeta* sp. (2)	*Rhododendron* sp. (2)	2 / 0
	Coniochaeta decumbens (8)	*Pinus pungens* (3)	2 / 1
		Pinus rigida (2)	1 / 1
		Rhododendron sp. (2)	1 / 1
		Rhododendron maximum (1)	1 / 0
Ascomycota; Diaporthales	Unknown Diaporthae (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Diaporthales; Erythrogloeaceae	*Dendrostoma castaneum* (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Dothideales; Dothideaceae	*Coniozyma* sp. (1)	*Rhododendron maximum* (1)	1 / 0
	Scleroconidioma sphagnicola (2)	*Pinus pungens* (2)	2 / 0
Ascomycota; Dothideales; Dothioraceae	*Sydowia polyspora* (14)	*Pinus pungens* (2)	1 / 1
		Pinus rigida (8)	2 / 6
		Rhododendron sp. (4)	1 / 3
Ascomycota; Dothideales; Saccotheciaceae	*Aureobasidium* sp. (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Eurotiales; Aspergillaceae	*Penicillium* sp. (1)	*Pinus pungens* (1)	1 / 0
Ascomycota; Helotiales; Heterosphaeriaceae	*Heterosphaeria* sp. (1)	*Pinus pungens* (1)	0 / 1
Ascomycota; Hypocreales; Hypocreales *incertae sedis*	*Cylindrium elongatum* (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Leotiales; Tympanidaceae	*Tympanis prunicola* (1)	*Pinus pungens* (1)	0 / 1
Ascomycota; Leotiomycetes *incertae sedis*	*Scytalidium flavobrunneum* (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Pezizales; Pyronemataceae	*Anthracobia* sp. (2)	*Pinus pungens* (1)	0 / 1
		Pinus rigida (1)	0 / 1
	Sphaerosporella sp. (2)	*Pinus pungens* (1)	0 / 1

		Pinus rigida (1)	0 / 1
Ascomycota; Pleosporales; Astrosphaeriellaceae	*Pithomyces chartarum* (1)	*Pinus rigida* (1)	0 / 1
Ascomycota; Pleosporales; Didymellaceae	*Didymella* sp. (1)	*Rhododendron* sp. (1)	0 / 1
	Epicoccum nigrum (1)	*Pinus pungens* (1)	0 / 1
	Phoma sp. (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Pleosporales; Pleosporaceae	*Alternaria alternata* (1)	*Rhododendron* sp. (1)	0 / 1
Ascomycota; Sordariales	Unknown Sordariomycete (1)	*Pinus* sp. (1)	0 / 1
Ascomycota; Sordariales; Diplogelasinosporaceae	*Diplogelasinospora grovesii* (1)	*Rhododendron* sp. (1)	1 / 0
Ascomycota; Sordariales; Schizotheciaceae	*Jugulospora rotula* (1)	*Pinus pungens* (1)	1 / 0
Ascomycota; Xylariales; Hypoxylaceae	*Annulohypoxylon truncatum* (1)	*Rhododendron maximum* (1)	0 / 1
Ascomycota; Xylariales; Sporocadaceae	*Pestalotiopsis chamaeropis* (4)	*Pinus rigida* (1)	0 / 1
		Rhododendron sp. (3)	1 / 2
	Pestalotiopsis pini (3)	*Rhododendron* sp. (3)	2 / 1
Ascomycota; Xylariales; Xylariaceae	*Biscogniauxia* sp. (1)	*Pinus rigida* (1)	1 / 0
	Rosellinia corticium (2)	*Rhododendron* sp. (1)	0 / 1
		Rhododendron maximum (1)	0 / 1
Basidiomycota; Corticiales; Corticiae	*Sistotrema oblongisporum* (1)	*Rhododendron* sp. (1)	1 / 0
Basidiomycota; Cystofilobasidiales; Mrakiaceae	*Tausonia pullulans* (1)	*Rhododendron* sp. (1)	1 / 0
Mucoromycota; Umbelopsidales; Umbelopsidae	*Umbelopsis nana* (1)	*Pinus pungens* (1)	1 / 0
N/A	Dark taxon #1 (1)	*Pinus* sp. (1)	0 / 1
N/A	Dark taxon #2 (1)	*Pinus pungens* (1)	1 / 0
N/A	Ariz:P155 (1)	*Rhododendron* sp. (1)	1 / 0

[a]Proportion of heat resistant/non-heat resistant individuals out of *n* individuals of a genus/species

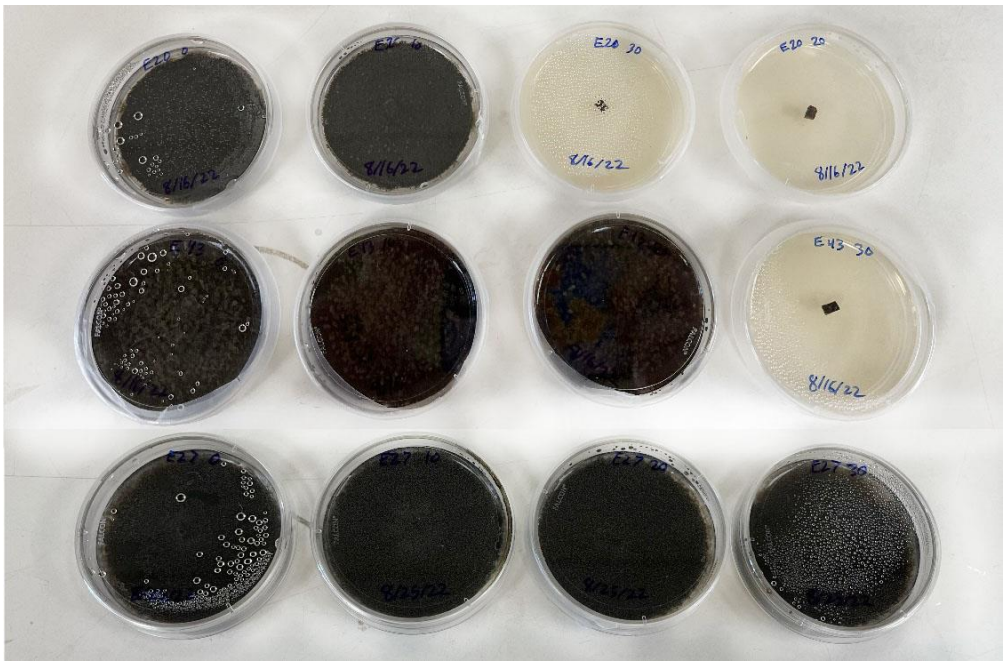

Figure 1. A range of endophyte growth patterns following heat shock. Each row consists of one endophyte isolate. From left to right, each column contains endophyte isolates heated for 0, 10, 20, and 30 minutes, respectively. The first two endophyte isolates were not heat resistant because they did not grow after 30 minutes of heating. However, the second isolate may be more heat resistant than the first because it grew after 20 minutes of heating where the first did not. The third isolate was heat resistant and grew quickly in all heating conditions.

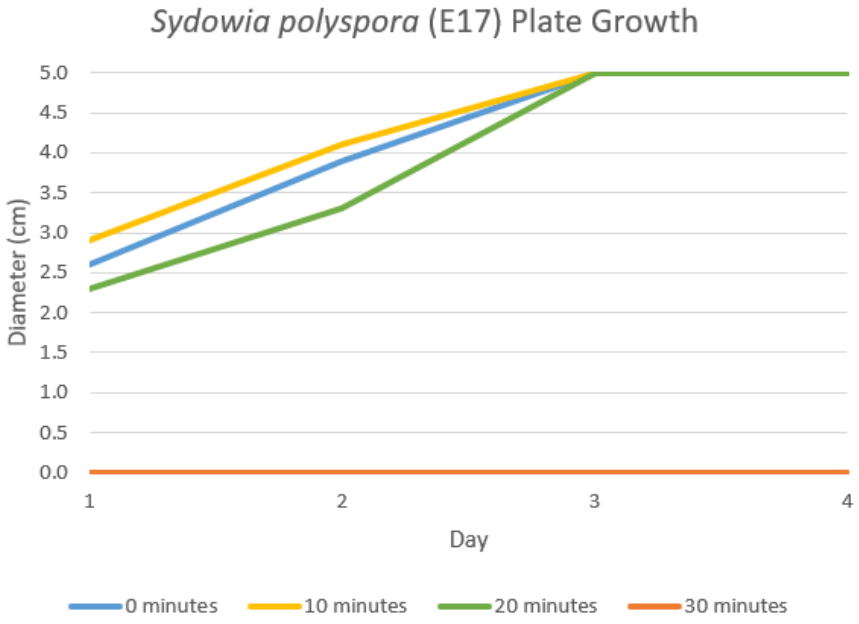

Figure 2. Growth progress of non-heat resistant *Sydowia polyspora* (endophyte isolate #17) after heat shock.

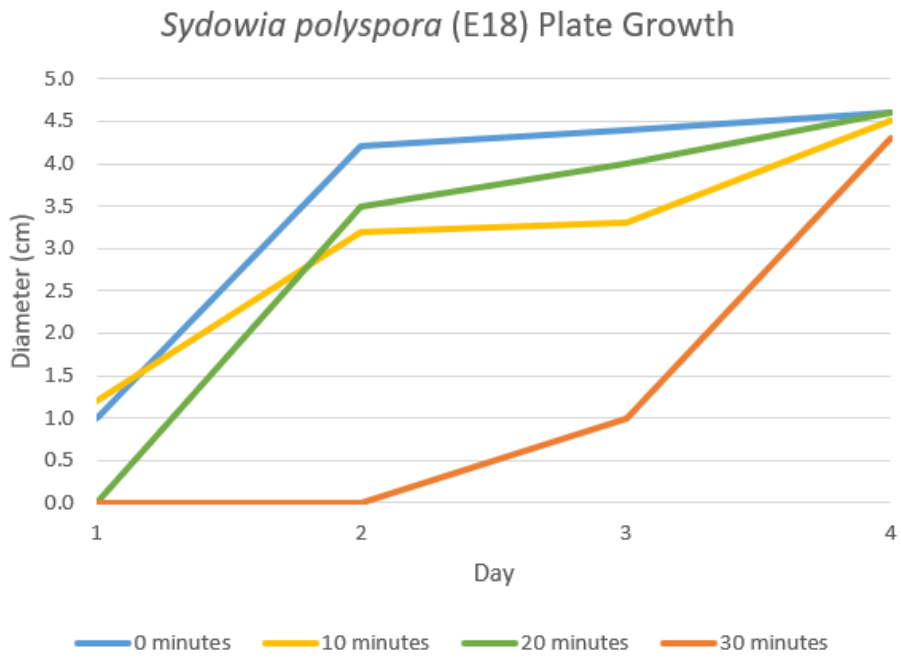

Figure 3. Growth progress of heat resistant *Sydowia polyspora* (endophyte isolate #18) after heat shock.

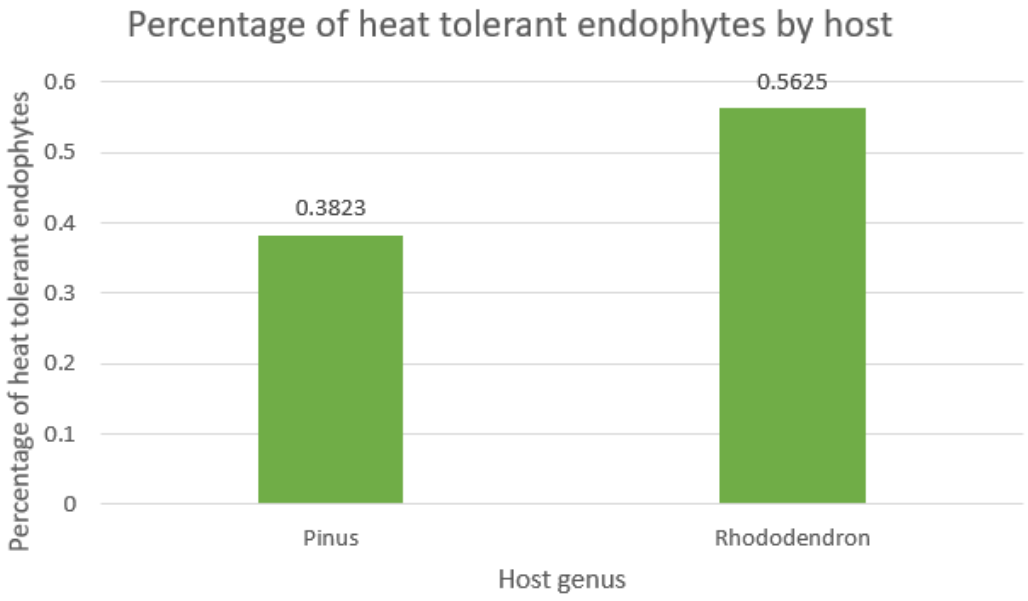

Figure 4. Proportion of heat resistant endophytes isolated from *Pinus* and *Rhododendron* hosts.

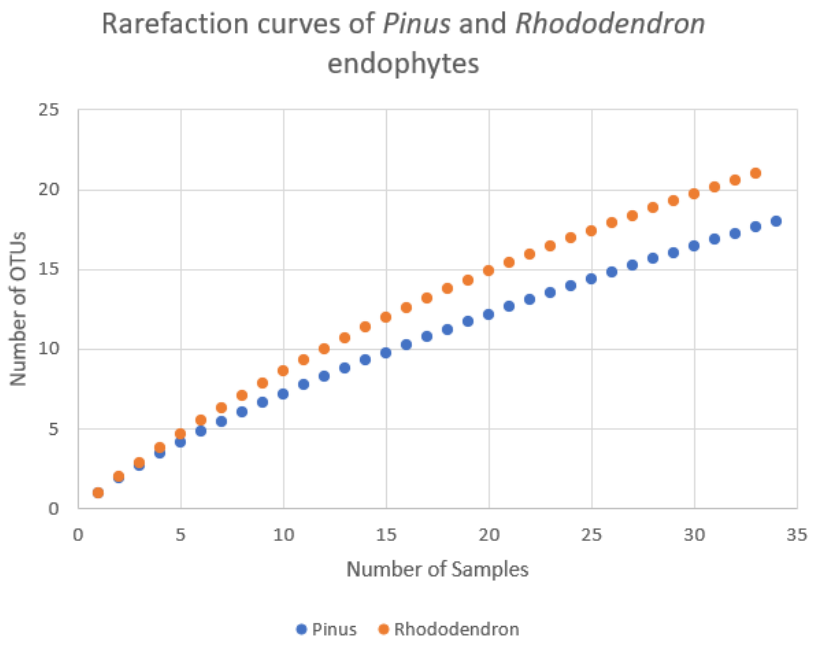

Figure 5. Rarefaction curves for *Pinus* and *Rhododendron* endophyte communities, modeling number of OTUs found in each host as a function of sampling effort.

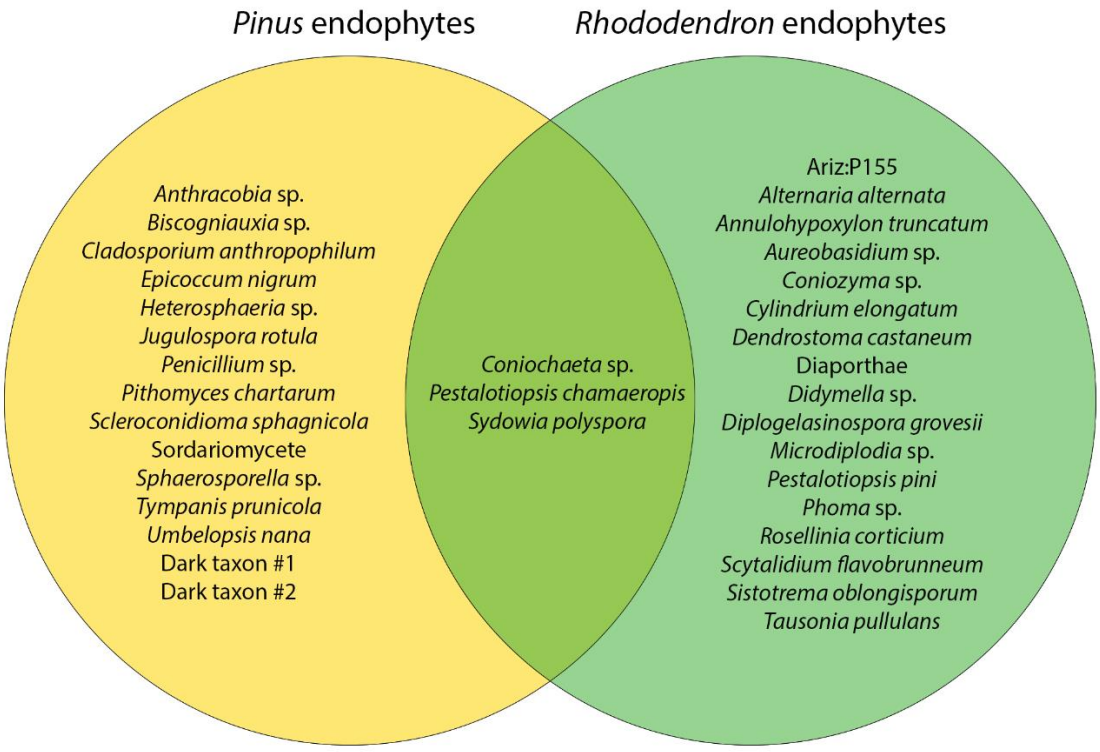

Figure 6. Taxa unique to and shared between *Pinus* and *Rhododendron* hosts at Chimney Tops 2 burn sites.

Discussion:

It would not be surprising if endophytic fungi of fire adapted plants also showed heat tolerance. Regular wildfires would be expected to select for heat resistant fungi in soils (a source inoculum of endophytic fungi) or inside fire-scorched vegetation. Unexpectedly, we found that less than half of the endophyte isolates we tested, both from pine (fire adapted) and *Rhododendron* (not fire adapted), were heat resistant. We also found that *Rhododendron* plants hosted significantly more heat tolerant endophytes than pine seedlings ($P<0.05$). Neither of these findings supported our original hypotheses. These results suggest that the distribution of post-fire successional endophytes at the Chimney Tops 2 burn sites may not be associated with host plant fire adaptation.

The rarefaction curves we created for the pine and *Rhododendron* endophyte communities (Fig. 5) show that, with the same amount of sampling effort, more endophyte species may be found in *Rhododendron* plants than in pine seedlings. We also found that *Rhododendron* plants had a higher alpha diversity of endophytes than pine seedlings. These results indicate that *Rhododendron* plants hosted a greater richness of endophyte species than pine seedlings. A variety of ecological factors can shape the diversity of endophyte communities, including host plant genotype (Alholm et al., 2002) and plant functional traits (Li et al., 2018; Peršoh, 2013).

One explanation for the greater proportion of heat resistant endophytes in *Rhododendron* and the greater overall diversity of *Rhododendron* endophyte species is that *Rhododendron* plants at the burn sites may have retained some of their pre-fire endophyte communities. It is possible that some foliar endophytes were able to survive the fire by living on in *Rhododendron* roots underground after the fire destroyed their above ground structures. The endophytes would still have experienced some heat shock in the *Rhododendron* roots; high intensity wildfires can heat soil to over 250°C at 10cm of depth and over 100°C as far as 22cm below the surface (Neary et al., 1999). Thus, there may have been selection for heat resistant endophytes in *Rhododendron* roots. After the fire, the *Rhododendron* regenerated from its roots, and its endophytes could have spread into its leaves. It is unlikely that the pine seedlings at the burn sites would have hosted as many endophytes as *Rhododendron* plants. Endophytes of woody plants are not usually vertically transmitted, so the successional pine seedlings would have had fewer or no endophyte species at the beginning of their growth than successional *Rhododendron* plants. It is possible that, if we had studied adult pine trees, we would have observed different proportions of heat resistant endophytes than we found in the present study. Sampling endophyte communities at the time of primary succession limits the breadth of community diversity we can observe in long-lived hosts like pine trees.

At the time of sampling in 2017, both *Rhododendron* and pine populated burn sites. However, within one year after sampling, most successional *Rhododendron* plants at the burn sites had died off (K. Hughes, personal communication, March 10, 2023). We found a high beta diversity of endophyte species between *Rhododendron* and pine communities, meaning

that *Rhododendron* hosted many endophyte species that were not found in pine, and vice versa. The death of *Rhododendron* plants in the years after the fire could bode poorly for the endophyte species unique to *Rhododendron*. Endophytes often exhibit host specificity, associating frequently or exclusively with certain families, genera, or species (Arnold, 2007; Petrini & Carroll, 1981). This means that fungal endophytes specific to *Rhododendron* hosts may decrease over time at burn sites dominated by fire adapted plants.

Both pine and *Rhododendron* hosted non-heat resistant endophytes. This indicates that some proportion of successional endophytes are non-heat resistant endophytes that have aerially dispersed from outside the burn area, survived in deeper soil layers, or entered burn sites by other means. Many pyrophilous species of fungi naturally occur as mycorrhizal symbionts, plant pathogens, soil saprophytes, and endophytes (Hughes et al., 2020a). Several species of pyrophilous fungi were isolated from the burn sites. We hypothesized that these pyrophilous fungi would be heat resistant because of their affinity for burned areas. In affiliation with the present study, Hughes et al. (2020a) documented the post-fire proliferation of *Sphaerosporella* spp. at Chimney Tops 2 burn sites and demonstrated that it was capable of both aerial and soil endophytic infection of germinating *Pinus pungens* seedlings. *Sphaerosporella* rapidly proliferates after wildfires (Hughes et al., 2020a), so we expected that the *Sphaerosporella* isolates tested in the present study would be heat resistant. We expected to see similar results from other pyrophilous genera like *Anthracobia* (Claridge et al., 2009; Fujimura et al., 2005; Hughes et al., 2020b) and *Coniochaeta* (Wicklow, 1975). However, we found that none of the *Anthracobia* or *Sphaerosporella* isolates we tested were heat resistant and only 70% of *Coniochaeta* isolates were heat resistant (Table 1). This suggests that pyrophilous fungi are not necessarily heat resistant and that heat resistant fungi are not necessarily pyrophilous. We note that neither *Sphaerosporella* nor *Anthracobia* are known to be endophytes (Hughes et al., 2020b), so their recovery as endophytes may indicate an early opportunistic infection of plants following fire rather than a sustained infection over time.

The fact that *some* but not *all* of the endophytes tested were heat resistant makes it difficult to draw substantive conclusions about why certain endophytes are or are not heat resistant. These results suggest that the burn sites and hosts are not definitively "heat tolerant" or "non-heat tolerant"; rather, each burn site and host is home to a mixture of heat resistant and non-heat resistant endophytes. One factor that may have influenced the unexpectedly low proportion of heat resistant endophytes in the GSMNP is fire suppression. Fire was suppressed in the park for most of the 20th century (Flatley et al., 2013), so it is possible that a greater number of heat resistant endophytes may be found at sites with more frequent fires. Carlsson et al. (2012) found that saprotrophic fungi endemic to regions with frequent fires are more heat tolerant than fungi found in locations without regular fire; the same pattern may hold true for fungal endophytes.

Since we did not find broader community patterns of heat resistance across hosts or burn sites, we expected that heat resistance would vary by species—that certain endophyte

species would be heat resistant and others would not be. However, we found that heat resistance varied intraspecifically for almost all of the endophytes for which multiple representatives of a species were tested (Table 1). Intraspecific variation in heat resistance was most evident among isolates of *Sydowia polyspora*, which was the species with the greatest abundance of isolates. Of the 14 *S. polyspora* isolates tested, 4 were heat resistant and 10 were not heat resistant. Figures 2 and 3 show the differences in heat tolerance and post-heat shock growth between two isolates of *S. polyspora*. It is possible that the putative intraspecific variation of heat tolerance in *S. polyspora* was caused by methodical errors such as contamination or varying ages of cultured specimens. However, nrITS sequencing found that all of the isolates of *S. polyspora* definitively belonged to the same taxon with little taxonomic noise. Thus, contamination is an unlikely cause of variability in the heat resistance of *S. polyspora* isolates. The age of the original endophyte cultures from which the isolates tested in the present study were subcultured could still have influenced heat resistance patterns in isolates. Our results indicate that heat resistance varies by genotype rather than by community or species. Some researchers have suggested that endophytes are more appropriately distinguished by genotype rather than by species concepts (Petrini et al., 1991). Heat resistant endophytes may share a common genotypic trait that non-heat resistant endophytes lack.

Two candidates for heat resistant traits that could vary by genotype are heat shock proteins and thermostable cellulases. Heat shock proteins are conserved proteins that repair cells and protect other proteins from denaturing under high heat (Jakob et al., 1993). Endophytes can use heat shock proteins to confer heat tolerance to their host plants (Baynes et al., 2012), so it is possible that the same proteins could play a role in an endophyte's own heat resistance (Carlsson et al., 2012). Thermostable cellulases can perform hydrolysis at high temperatures, meaning that an endophyte with thermostable cellulases could break down complex carbohydrates for consumption under heat shock. In one study of the effect of heat shock on cellulase efficiency, *Penicillium* isolates were heated at 60°C for 4.5 hours and maintained cellulase activity following heat shock (Yadav et al., 2022). The *Penicillium* isolate tested in the present study was found to be heat resistant (Table 1); this suggests a promising direction for further study of cellulase thermotolerance as an indicator of overall heat tolerance in an endophyte.

It is also possible that epigenetic effects—environmentally-influenced, heritable changes in gene function that do not alter DNA sequences (Dupont et al., 2009)—could cause intraspecific variation in heat resistance. It has been hypothesized that fungal endophytes can cause epigenetic changes in stress tolerance of their host plants; DNA methylation, a common mechanism of epigenetic change, has been observed in drought- and salt-stressed plants with endophytic symbionts (Hubbard et al., 2013). Woodward et al. (2012) even suggest that the very phenomenon of endophytic symbiosis is an epigenetic effect because endophytes alter plant gene expression and are often vertically transmitted to offspring. Epigenetics is a burgeoning topic in biology. More research is needed to understand how

biotic and abiotic environmental factors influence epigenetic change and how to detect such changes.

Limitations:

The primary limitation of the present study was a lack of replicates. For each endophyte isolate tested, only one replicate was used for each experimental heating condition (0, 10, 20, or 30 minutes of heating). This means that the response to heat shock we observed for each isolate was highly subject to idiosyncrasies of individual samples or methodical errors. Using multiple replicates for each experimental condition would offer more conclusive insight to each isolate's heat resistance. Working with more recent endophyte cultures could affect our findings as well; though they were subcultured many times between initial collection and the present study, the endophyte isolates tested in this study originated from samples collected in 2017. Future studies should test heat resistance in endophyte isolates soon after a burn site is accessible and employ robust replication in order to produce more reliable results.

Neither of the rarefaction curves of pine and *Rhododendron* endophyte community diversity have reached their asymptote. This means that more species richness could be found in both communities with more sampling effort. Further sampling and testing could reveal more information about patterns in local endophyte heat resistance. Additionally, it is likely that the present study underestimates the true diversity of post-fire endophyte communities because some endophytes cannot be cultured in a lab. Possible reasons for an endophyte's inability to be cultured include specialized host ranges, low competitive ability when growing on non-host substrate, or unique life history traits. It is hypothesized that a large portion of endophyte diversity lies in these unculturable endophytes (Arnold & Lutzoni, 2007). Barring advancements in endophyte culturing methods, it is likely that further study of endophyte diversity will continue to underestimate the true breadth of endophyte communities.

Implications:

The present study contributes to two fields that are gaining more attention with the progression of global warming: fungal epidemiology and fire ecology.

As global temperatures increase, all organisms must reckon with the need to withstand higher temperatures. Fungi are becoming more heat resistant as thermal selection favors those that can survive heat shock and sustained heat. This creates a novel opportunity for more fungi to become pathogenic to mammals, including humans. When a fungus develops heat tolerance in its native environment, it may also be able to thrive in locations that were previously inhospitable due to high temperatures (Casadevall et al., 2019). Normally, humans and other mammals are resistant to fungal pathogens because their body temperatures are too high for most fungal pathogens to survive (Robert & Casadevall, 2009). However, global warming can outfit fungi with the heat tolerance needed to successfully infect mammals. Over the next few decades, it is likely that we will see an increase in fungal

infections, possibly leading to epidemics (Vora, 2023). Two notable, heat resistant fungal pathogens that have already spread through human populations are *Cryptococcus deneoformans* and *Candida auris*. *Cryptococcus deneoformans* is a fungal pathogen that can infect humans through a wide variety of environmental reservoirs. Gusa et al. (2023) found that spontaneous genetic mutations in *C. deneoformans* increase fivefold when the fungus was exposed to 37°C/98°F temperatures. This means that *C. deneoformans* could evolve concerning adaptations like long term heat tolerance and antibiotic resistance more rapidly than expected. *Candida auris* is a yeast that has been pathogenic in humans for over a decade. In March 2023, an outbreak of *C. auris* spread through hospitals across the United States (Vora, 2023). It is hypothesized that this sudden surge of *C. auris* infections was driven by adaptations to high temperatures in response to climate change. Casadevall et al. (2023) even go so far as to say that *C. auris* is the first example of a pathogenic fungus to emerge from global warming. Fungal epidemiology is a field in urgent need of innovation. There are currently no vaccines for fungal pathogens, and it is very difficult to treat fungal infections because of the similarities between fungal and animal cells (Vora, 2023). Further research into endophyte heat tolerance would contribute to the burgeoning body of work focusing on climate-driven heat adaptation in fungi.

Another field of research gaining ever more relevancy is fire ecology. Global warming is increasing the frequency and intensity of wildfires (Neary et al., 1999). It is more important than ever to understand how ecological communities respond to changing fire regimes, especially in regions with naturally low or suppressed fire frequency since they stand to undergo the most change as fire frequency increases (Kelly et al., 2020). As fires increase in frequency and severity, species diversity in post-fire ecosystems will become increasingly different from pre-fire communities (Whitman et al., 2019). Fires are already creating novel ecosystems in their wake, and they will continue to do so for the foreseeable future. It is necessary for ecologists to understand how these fires reshape community dynamics, for better or for worse. Some species may benefit from fires; for example, fire can increase populations of previously rare, heat resistant species that prevail over their non-heat resistant peers after a fire (Carlsson et al., 2012). However, increasing fires can lead to population decline or even extirpation for vulnerable species. Even heat resistant fungi may be vulnerable to post-fire decline. The heat resistant *Rhododendron* endophytes at the Chimney Tops 2 burn sites prevailed through the fire, but likely met their demise within a year after the fire as their hosts died out. There is still much to understand about how fires benefit and harm ecological communities. The present study is the first to examine community structures of successional endophytes at moderate and high intensity burn sites. More research is necessary to understand the full scope of endophyte heat resistance and response to fire. Future research should investigate prevalence of endophytic heat resistance in unburned ecosystems or areas with frequent fires, genotypic traits indicative of heat resistance, and environmental factors that can influence heat resistance.

Literature Cited:

Alholm, J. U., Helander, M., Henriksson, J., Metzler, M., & Saikkonen, K. (2002). Environmental conditions and host genotype direct genetic diversity of *Venturia ditricha*, a fungal endophyte of birch trees. *Evolution*, *56*(8), 1566-1573. https://doi.org/10.1554/0014-3820(2002)056[1566:ECAHGD]2.0.CO;2

Ali, A. H., Abdelrahman, M., Radwan, U., El-Zayat, S., & El-Sayed, M. A. (2018). Effect of *Thermomyces* fungal endophyte isolated from extreme hot desertadapted plant on heat stress tolerance of cucumber. *Applied Soil Ecology*, *124*, 155-162. https://doi.org/10.1016/j.apsoil.2017.11.004

Anderson, Michelle D. (2008). *Rhododendron maximum*. Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. https://www.fs.usda.gov/database/feis/plants/shrub/rhomax/all.html

Arnold, A. E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews*, *21*, 51-66. https://doi.org/10.1016/j.fbr.2007.05.003

Arnold, A. E., & Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? *Ecology*, *88*(3), 541-549. https://doi.org/10.1890/05-1459

Baynes, M., Newcombe, G., Dixon, L., Castlebury, L., & O'Donnell, K. (2012). A novel plant-fungal mutualism associated with fire. *Fungal Biology*, *116*, 133-144. https://doi.org/10.1016/j.funbio.2011.10.008

Carlsson, F., Edman, M., Holm, S., Eriksson, A.-M., & Jonsson, B. G. (2012). Increased heat resistance in mycelia from wood fungi prevalent in forests characterized by fire: A possible adaptation to forest fire. *Fungal Biology*, *116*, 1025-1031. https://doi.org/10.1016/j.funbio.2012.07.005

Casadevall, A., Kontoyiannis, D. P., & Robert, V. A. (2019). On the emergence of *Candida auris*: Climate change, azoles, swamps, and birds. *mBio*, *10*(4). https://doi.org/10.1128/mBio.01397-19

Claridge, A. W., Trappe, J. M., & Hansen, K. (2009). Do fungi have a role as soil stabilizers and remediators after forest fire? *Forest Ecology and Management*, *257*(3), 1063-1069. https://doi.org/10.1016/j.foreco.2008.11.011

Dunn, P.H., Barro, S.C., & Poth, M. (1985). Soil moisture affects survival of microorganisms in heated chaparral soil. *Soil Biology and Biochemistry*, *17*(2), 143-148. https://doi.org/10.1016/0038-0717(85)90105-1

Dupont, C., Armant, D. R., & Brenner, C. A. (2009). Epigenetics: Definition, mechanisms and clinical perspective. *Seminars in Reproductive Medicine*, *27*(5), 351-357. https://doi.org/10.1055/s-0029-1237423

El-Abyad, M. S. H., & Webster, J. (1968). Studies on pyrophilous discomycetes: I. Comparative physiological studies. *Transactions of the British Mycological Society*, *51*, 353-367. https://doi.org/10.1016/S0007-1536(68)80002-6

Flatley, W. T., Lafon, C. W., Grissino-Mayer, H. D., & LaForest, L. B. (2013). Fire history, related to climate and land use in three southern Appalachian landscapes in the eastern United States. *Ecological Applications*, *23*(6), 1250-1266. https://doi.org/10.1890/12-1752.1

Fujimura, K. E., Smith, J. E., Horton, T. R., Weber, N. S., & Spatafora, J. W. (2005). Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. *Mycorrhiza*, *15*(2), 79-86. https://doi.org/10.1007/s00572-004-0303-8

Great Smoky Mountains National Park (2022, October 21). *Blooming Shrubs*. Great Smoky Mountains National Park (U.S. National Park Service). https://www.nps.gov/grsm/learn/nature/blooming-shrubs.htm

Gusa, A., et al. (2023). Genome-wide analysis of heat stress-stimulated transposon mobility in the human fungal pathogen *Cryptococcus deneoformans*. *Proceedings of the National Academy of Sciences*, *120*(4). https://doi.org/10.1073/pnas.2209831120

Hirsch, G., & Braun, U. (1992). Communities of parasitic microfungi. In W. Winterhoff (Ed.), *Fungi in vegetation science* (pp. 225-250). Springer Science+Business Media Dordrecht. https://doi.org/10.1007/978-94-011-2414-0_8

Hubbard, M., Germida, J. J., & Vujanovic, V. (2013). Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second-generation seed viability. *Journal of Applied Microbiology*, *116*, 109-122. https://doi.org/10.1111/jam.12311

Hughes, K. W., Case, A., Matheny, P. B., Kivlin, S., Petersen, R. H., Miller, A. N., & Iturriaga, T. (2020). Secret lifestyles of pyrophilous fungi in the genus *Sphaerosporella*. *American Journal of Botany*, *107*(6), 876-885. https://doi.org/10.1002/ajb2.1482

Hughes, K. W., Matheny, P. B., Miller, A. N., petersen, R. H., Iturriaga, T., Johnson, K. D., Methven, A. S., Raudabaugh, D. B., Swenie, R. A., & Bruns, T. D. (2020). Pyrophilous fungi detected after wildfires in the Great Smoky Mountains National Park expand known species ranges and biodiversity estimates. *Mycologia*, *112*(4), 677-698. https://doi.org/10.1080/00275514.2020.1740381

Jakob, U., Gaestel, M., Engel, K., & Buchner, J. (1993). Small heat shock proteins are molecular chaperones. *The Journal of Biological Chemistry*, *268*(3), 1517-1520. https://doi.org/10.1016/S0021-9258(18)53882-5

Jesenska, Z., Pieckova, E., & Bernat, D. (1993). Heat resistance of fungi from soil. *International Journal of Food Microbiology*, *19*(3), 187-192. https://doi.org/10.1016/0168-1605(93)90076-S

Keeley, J. E. (2012). Ecology and evolution of pine life histories. *Annals of Forest Science*, *69*, 445-453. https://doi.org/10.1007/s13595-012-0201-8

Kelly, L. T., Giljohann, K. M., Duane, A., Aquilué, N., Archibald, S., Batllori, E., Bennett, A. F., Buckland, S. T., Canelles, Q., Clarke, M. F., Fortin, M.-J., Hermoso, V., Herrando, S., Keane, R. E., Lake, F. K., McCarthy, M. A., Morán-Ordóñez, A., Parr, C. L., Pausas, J. G., . . . Brotons, L. (2020). Fire and biodiversity in the Anthropocene. *Science*, *370*. https://doi.org/10.1126/science.abb0355

Kuo, H.-C., Hui, S., Choi, J., Asiegbu, F. O., Valkonen, J. P. T., & Lee, Y.-H. (2014). Secret lifestyles of *Neurospora crassa*. *Scientific Reports*, *4*(5135). https://doi.org/10.1038/srep05135

Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276-3278. https://doi.org/10.1093/bioinformatics/btu531

Li, Y., Wu, X., Chen, T., Wang, W., Liu, G., Zhang, W., Li, S., Wang, M., Zhao, C., Zhou, H., & Zhang, G. (2018). Plant phenotypic traits eventually shape its microbiota: A common garden test. *Frontiers in Microbiology*, *9*(2479). https://doi.org/10.3389/fmicb.2018.02479

Matheny, P. B., Swenie, R. A., Miller, A. N., Petersen, R. H., & Hughes, K. W. (2018). Revision of pyrophilous taxa of Pholiota described from North America reveals four species—*P. brunnescens, P. castanea, P. highlandensis*, and *P. molesta*. *Mycologia*, *110*(6), 997-1016. https://doi.org/10.1080/00275514.2018.1516960

Miller, A. N., Raudabaugh, D. B., Iturriaga, T., Matheny, P. B., Petersen, R. H., Hughes, K. W., Gube, M., Powers, R. A., James, T. Y., & O'Donnell, K. (2017). First report of the post-fire morel *Morchella exuberans* in eastern North America. *Mycologia*, *109*(5), 710-714. https://doi.org/10.1080/00275514.2017.1408294

Nagabhyru, P., Dinkins, R. D., & Schardl, C. L. (2022). Transcriptome analysis of *Epichloë* strains in tall fescue in response to drought stress. *Mycologia, 114*(4), 697-712. https://doi.org/10.1080/00275514.2022.2060008

Neary, D. G., Klopatek, C. C., DeBano, L. F., & Ffolliott, P. F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management*, *122*, 51-71. https://doi.org/10.1016/S0378-1127(99)00032-8

Peršoh, D. (2013). Factors shaping community structure of endophytic fungi--evidence from the *Pinus-Viscum*-system. *Fungal Diversity*, *60*, 55-69. https://doi.org/10.1007/s13225-013-0225-x

Petrini, O., Andrews, J. H., & Hlrano, S. S. (1991). Fungal Endophytes of Tree Leaves. In *Microbial Ecology of Leaves* (pp. 179-197). Springer-Verlag New York Inc. https://doi.org/10.1007/978-1-4612-3168-4

Petrini, O., & Carroll, G. (1981). Endophytic fungi in foliage of some Cupressaceae in Oregon. *Canadian Journal of Botany*, *59*, 629-636. https://doi.org/10.1139/b81-089

Raudabaugh, D. B., Matheny, P. B., Hughes, K. W., Iturriaga, T., Sargent, M., & Miller, A. N. (2020). Where are they hiding? Testing the body snatchers hypothesis in pyrophilous fungi. *Mycologia*, *43*. https://doi.org/10.1016/j.funeco.2019.100870

Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., & Henson, J. M. (2002). Thermotolerance generated by plant/fungal symbiosis. *Science*, *298*(5598), 1581.

Robert, V. A., & Casadevall, A. (2009). Vertebrate endothermy restricts most fungi as potential pathogens. *The Journal of Infectious Diseases*, *200*, 1623-1626. https://doi.org/10.1086/644642

Rodriguez, R. J., White, J. F., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: Diversity and functional roles. *New Phytologist*, *182*(2), 314-330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Saikkonen, K., Wali, P., Helander, M., & Faeth, S. H. (2004). Evolution of endophyte–plant symbioses. *Trends in Plant Science*, *9*(6), 275-280. https://doi.org/10.1016/j.tplants.2004.04.005

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V. A., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, *109*(16), 6241-6246. https://doi.org/10.1073/pnas.1117018109

Seaver, F. J. (1909). Studies in pyrophilous fungi--I. The occurrence and cultivation of *Pyronema*. *Mycologia*, *1*(4), 131-139. https://doi.org/10.1080/00275514.1909.12020584

Stone, J. K., Polishook, J. D., & White, J. F. (2004). Endophytic Fungi. In G. M. Mueller, G. F. Bills, & M. S. Foster (Eds.), *Biodiversity of fungi : Inventory and monitoring method* (pp. 241-269). Academic Press. https://doi.org/10.1016/B978-012509551-8/50015-5

Vora, Neil (2023, April 2). "'The Last of Us' Is Right. Our Warming Planet Is a Petri Dish." *The New York Times*. https://www.nytimes.com/2023/04/02/opinion/the-last-of-us-fungus-climate-change.html?searchResultPosition=2

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., & Parisien, M.-A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biology and Biochemistry*, *138*. https://doi.org/10.1016/j.soilbio.2019.107571

Whittaker, R. H. (1972). Evolution and measurement of species diversity. *Taxon*, *21*(2/3), 213-251. https://doi.org/10.2307/1218190

Wicklow, D. T. (1975). Fire as an environmental cue initiating ascomycete development in a tallgrass prairie. *Mycologia*, *67*(4), 852-862. https://doi.org/10.2307/3758344

Wilson, D. (2000). Ecology of Woody Plant Endophytes. In C. W. Bacon & J. White (Eds.), *Microbial Endophytes* (1st edition ed., pp. 389-420). CRC Press. https://doi.org/10.1201/9781482277302

Witfield, F., Begerow, D., & Guerreiro, M. A. (2021). Improved strategies to efficiently isolate thermophilic, thermotolerant, and heat-resistant fungi from compost and soil. *Mycological Progress*, *20*, 325-339. https://doi.org/10.1007/s11557-021-01674-z

Woodward, C., Hansen, L., Beckwith, F., Redman, R.S., & Rodriguez, R.J. (2012). Symbiogenics: An epigenetic approach to mitigating impacts of climate change on plants. *HortSci*, *47*(6), 699-703. https://doi.org/10.21273/HORTSCI.47.6.699

Yadav, R., Vasundhara, M., Rajamani, T., Suryanarayanan, T. S., & Reddy, S. M. (2022). Isolation and characterization of thermostable and alkali-tolerant cellulase from litter endophytic fungus *Bartalinia pondoensis*. *Folia Microbiologica*, *67*(6), 955-964. https://doi.org/10.1007/s12223-022-00991-4