

Genomic variation in *Sydowia polyspora* in relation to geography and host symptom severity

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Abstract

Background. *Sydowia polyspora* is a widespread conifer-associated fungus with ecological roles that vary among host and disease contexts. However, how its genomic variation corresponds to geography and host symptom severity remains poorly understood. We examined whether genomic differentiation among *S. polyspora* isolates recovered from symptomatic *Pinus taeda* varied between geographic regions and among host symptom-severity classes.

Methods. We analyzed whole-genome sequence data from 25 *S. polyspora* isolates. Genome-wide single-nucleotide polymorphisms were used to evaluate population structure through principal component analysis and quantify differentiation among geographic and symptom-severity groups using F_{ST} . Nucleotide diversity and Tajima's D were estimated in 5-kb windows to characterize genomic variation within groups.

Results. Isolates overlapped broadly in principal component analysis, but pairwise comparisons revealed geographically variable differentiation. Differentiation between high- and low/moderate-severity groups was minimal in northern Alabama, whereas the

corresponding southern comparison showed broadly distributed genomic differentiation (mean $F_{ST} = 0.37$). Northern and southern isolates exhibited more modest differentiation (mean $F_{ST} = 0.11$). Nucleotide diversity and Tajima's D varied across the genome but showed substantial overlap among geographic and symptom-severity groups. Genomic differentiation between symptom-severity groups was evident in southern but not northern Alabama, suggesting that associations between *S. polyspora* genetic background and host symptom severity may vary geographically.

Keywords: disease ecology, fungal ecology, forest pathology, needle blight, *Pinus teada*

Introduction

Variation among individuals and populations of plant-associated fungi can have important consequences for disease ecology. Genetically differentiated fungal populations may vary in traits related to host colonization, pathogenicity, virulence, and host specialization, while population structure can also reflect differences in dispersal, introduction history, and local evolutionary processes (Grünwald et al., 2016a). Genetic variation can, therefore, influence how fungi interact with their hosts and how fungal populations respond to changing ecological conditions. In forest disease systems, such variation is particularly relevant because fungal populations can occur across heterogeneous landscapes in which host condition, environmental conditions, and pathogen population history vary simultaneously (Plantegenest et al., 2007; Webb et al., 2006).

Population genomics provides a means of determining how fungal genomic variation is structured across landscapes and host contexts. Population structure in plant-associated fungi

can reflect geographic isolation, dispersal, introductions, recombination, host association, and other aspects of evolutionary and demographic history (Grünwald et al., 2016a; McDonald & Linde, 2002). For example, *Dothistroma septosporum* exhibits substantial population structure associated with geography and introduction history, while genomic analyses of *Lecanosticta acicola* have revealed divergent lineages and admixture where those lineages co-occur (Barnes, Walla, et al., 2014; Marcet-Houben Marina et al., 2024). However, geographic population structure does not necessarily correspond to variation in host symptom expression but rather a quantitative outcome shaped by pathogen variation, host genotype and host-pathogen interactions (Amezrou et al., 2024; Sacristán & García-Arenal, 2008; Williams-Blangero et al., 2012). Examining genomic variation across both geographic and host symptom-severity groups can therefore provide an initial assessment of whether patterns of fungal differentiation vary across these contexts..

Associations between fungal variation and host symptom severity may not be limited to the primary causal pathogen. Foliar diseases often occur within a broader assemblage of microorganisms (Kashyap et al., 2026; Meinecke et al., 2024), and associated fungi may increase or decrease symptom severity through interactions with the pathogen, host, or other members of the foliar microbiome (Ridout & Newcombe, 2015). Such effects have been demonstrated experimentally in conifer needle diseases. For example, fungi associated with *Pinus ponderosa* altered the severity of *Dothistroma* needle blight, with some reducing disease severity and others acting as pathogen enablers (Ridout & Newcombe, 2015). In this case, *Sydowia polyspora* increased disease severity in that system despite not being the causal

69 agent. Thus, relationships between fungal variation and host symptom severity may extend
70 beyond the causal pathogen to other fungi associated with diseased tissues.

71
72 *Sydowia polyspora* is a widespread conifer-associated fungus with notably variable ecological
73 associations. This fungus has been recovered from both symptomatic and asymptomatic
74 tissues and has been described as an endophyte, saprophyte, opportunistic pathogen, and
75 disease modifier depending on host and environmental context (Ridout & Newcombe, 2015,
76 2018; Silva et al., 2020; Talgø et al., 2010). Experimental studies have demonstrated
77 pathogenic effects in some conifer systems, while its recovery from asymptomatic tissues and
78 alongside other needle-associated fungi suggests that its ecological associations vary among
79 host and disease contexts (Pan et al., 2018). Despite this ecological breadth, we do not yet
80 know how genetic variation within *S. polyspora* is structured across landscapes and host
81 contexts. Understanding this variation is particularly relevant for a fungus with such variable
82 ecological associations because geographically differentiated genetic backgrounds may occur
83 independently of variation in host symptom expression.

84
85 In this study, we examined *P. teada* exhibiting needle-blight symptoms that varied in severity
86 across sampled trees. We combined culture-based characterization of fungi recovered from
87 affected needles with whole-genome sequencing of *S. polyspora* to characterize genomic
88 variation in relation to geography and host symptom severity. We first characterized
89 culturable fungi recovered across the symptom-severity gradient to identify taxa consistently
90 recovered from affected needles. We then focused on *S. polyspora*, the most consistently
91 recovered fungal taxon, to characterize genome-wide genetic diversity and ask whether

isolates exhibited population structure among sampled geographic regions and genomic differentiation among symptom-severity classes within regions. These analyses provide an initial genetic characterization of *S. polyspora* associated with symptomatic *P. teada* and evaluate whether patterns of genomic differentiation vary across geographic and host symptom-severity contexts.

Methods

Sample collection and culturing

Needle samples exhibiting blight disease symptoms were collected from *P. teada* in Alabama, specifically in Washington and Cullman Counties between March and August in 2023 and in Winston and Macon counties between March and August in 2024 (Supplementary Figure 1). Each sampling site (N=22) consisted of a central plot and three additional subplots located approximately 120° apart around the central plot, resulting in a total of 88 plots (Supplementary Figure 1). Collected samples were placed in paper bags, transported on ice to the laboratory, and stored at -20°C. For each sampled tree, we also recorded symptom severity, which was based on the percentage of symptomatic needles on the bottom, middle, and top of each tree following forest health practitioner rating guidelines (Bergdahl et al., 2022). For this study, symptoms severity for each sampled tree was estimated as 0 to 33% symptomatic needles (low), > 33% and up to 66% symptomatic needles (moderate), and > 66% symptomatic needles (high). All needle samples were processed within three days of collection for culturing and isolation following methods described in (Folorunso et al., 2025). Asymptomatic needles were also collected and cultured using the same approach; however, these samples did not yield colonies exhibiting the morphological characteristics targeted for

subsequent isolation and identification and were therefore not included in further analyses.

Four culture replicates were prepared from each processed needle. Colonies exhibiting acervuli with olive green to dark green or brown-black growth were preferentially subcultured, exhibiting characteristics commonly associated with needle-associate fungi (Supplementary Figure 2). These selected colonies were transferred to mini sleeves (30 mm) and incubated for one to two weeks at 25°C to obtain pure cultures while minimizing cross-contamination among isolates (Supplementary file 2) (Mullett et al., 2018; Van Der Nest et al., 2019).

To identify fungal taxa recovered through culture-based isolation, we extracted DNA from all subcultured isolates using a phenol-chloroform extraction method. Mycelium was homogenized in 600 µL lysis buffer and treated with 2.5 µL proteinase K. DNA was extracted using phenol:chloroform:isoamyl alcohol followed by RNase treatment and ethanol precipitation. The internal transcribed spacer (ITS) region was amplified using the universal fungal primers ITS1 and ITS4 (White et al., 1990), and PCR products were then separated on a 2% agarose gel to confirm amplification and expected amplicon size. The resulting amplicons were Sanger sequenced using the universal fungal primer ITS5 (White et al., 1990) at the Genomics Core Lab at University of Alabama Birmingham for species identification. Sequence chromatograms were quality-trimmed by sangerseq in R with allowing up to two mismatches, and reads shorter than 100bp after trimming to be excluded, and resulting sequences were queried against NCBI GenBank databases using BLASTn (v2.15.0+). These identifications were used to characterize fungal taxa recovered from symptomatic needles and

to identify taxa consistently recovered from symptomatic needles for subsequent genomic analysis.

We used fungal species identified from BLAST analysis to characterize fungal taxa recovered from symptomatic *P. teada* needles and identify taxa consistently recovered across the needle-blight symptom-severity gradient. All statistical analyses were conducted in R, using individual trees as the primary unit of analysis for cultured isolates. For each taxon, prevalence was calculated as the proportion of sampled trees from which that fungal taxon was recovered. Species richness was calculated as the number of unique fungal taxa recovered from each tree. The relationship between species richness and symptom severity was evaluated using Spearman's rank correlation coefficient, with symptom severity represented by ordinal severity category. Taxon prevalence and species richness were also summarized by region and symptom-severity category. These analyses were used to identify consistently recovered fungal taxa and establish the ecological context for subsequent population genomic analysis of *S. polyspora*.

Whole genome sequencing and analyses

To characterize genomic variation in *S. polyspora*, we extracted high-molecular weight DNA from recovered *S. polyspora* isolates using a modified CTAB protocol (Folorunso et al., 2025). DNA quality and quantity were assessed with a Quantus Fluorometer. Genomic DNA was prepared for long-read sequencing using the Oxford Nanopore Native Barcoding Kit 24 V14 (SQK-NBD114.24) following the manufacturer's protocol and sequenced libraries on a MinION device. Genomic DNA libraries were barcoded using Native Barcodes (NB01–24)

and ligated to Native Adapters using NEBNext Quick Ligation Reaction Buffer (5×) and 5 µL Quick T4 DNA Ligase. The pooled library was cleaned using Long Fragment Buffer and the final library was loaded onto a SpotON flow cell and sequenced for approximately three days. Raw reads were basecalled and demultiplexed simultaneously using Dorado (v 0.7.1) with basecalling model of super-accuracy simplex model. Resulting reads were quality assessed with FASTQC (Simon, et al., 2010) and summarized with MultiQC (Ewels et al., 2016). High-quality reads were assembled *de novo* assembled using Flye v.2 (Kolmogorov et al., 2019) to generate contiguous genome sequences and polished with Medaka v.3 (Wright & Wykes, 2023) . Assembly completeness was assessed with BUSCO (v5.4.0) (Benchmarking Universal Single-Copy Orthologs) (Simão et al., 2015) using the Dothideomycetes reference lineage.

To identify genome-wide variation among *S. polyspora* isolates , we generated a reference-based SNP dataset. Assemblies were evaluated using total assembly length, GC content, N50, and contig number. The isolate with the highest BUSCO completeness was selected as the reference for read mapping and variant discovery across all remaining same species isolates. Reads from the remaining *S. polyspora* isolates were aligned to this reference genome using minimap2 (Li, 2018). Alignment files were sorted and indexed using SAMtools, and SNPs were called using the bcftools mpileup and bcftools call pipeline (Danecek et al., 2021). SNPs were quality-filtered prior to population analyses by filtering out low sites with Phred quality < 20 per sample or read depth <10 per isolates from the VCF file using bcftools.

Population genomic analyses were conducted using isolates meeting a minimum BUSCO completeness threshold of >50% (N = 25). To evaluate genomic variation in relation to geography and symptom severity, isolates were assigned to groups based on region and symptom-severity category: North High (n = 3), North Low/Moderate (n = 8), South High (n = 8), and South Low/Moderate (n = 3). Isolates were also grouped by geographic region alone (North, N=11 ; South, N=11 ; outside Alabama, OA, N=3) for regional comparisons of genomic diversity. These statistics were calculated from filtered SNP datasets using VCFtools v0.1.17 (Danecek et al., 2011)

We evaluated broad-scale population structure using principal component analysis (PCA) following linkage-disequilibrium pruning in PLINK v1.9 (Purcell et al., 2007). To quantify genomic differentiation among geographic and symptom-severity groups, pairwise Weir and Cockerham F_{ST} values we used VCFtools v0.1.17 (Danecek et al., 2011). Window-based F_{ST} estimates were summarized across each pairwise comparison to characterize the magnitude and genomic distribution of differentiation among groups. To characterize within-group genomic diversity, nucleotide diversity and Tajima's D were calculated in 5-kb windows using VCFtools (version, citation). For each statistic, window-based values were summarized across the genome and within predefined geographic and symptom-severity groups to characterize genomic heterogeneity in diversity and allele-frequency patterns. Analyses were conducted on the Easley high-performance computing cluster at Auburn University and visualized in R using the tidyverse and ggplot2 packages (Wickham et al., 2019; Wickham & Sievert, 2009).

Results

Across all cultured needle samples, 169 fungal isolates were identified by BLAST, representing 60 putative species, 46 genera, and 19 orders (Figure 1A). Recovered fungi included taxa within *Rhizosphaera*, *Alternaria*, *Pestalopsis*, *Talaromyces*, *Xylaria*, *Aequabiliella*, *Aureobasidium*, and *Cladosporium*, among others (see Supplementary Table 1 for the complete list of recovered taxa and their occurrence across sampled trees). Prevalence patterns varied among taxa and symptom-severity classes, with many taxa detected A minantly or exclusively within a single severity class (Figure 1A). In contrast, *S. polyspora* was consistently prevalent across low-, moderate-, and high-severity trees (60%, 50%, and 62%, respectively), distinguishing it from most other recovered taxa. Species richness increased significantly with symptom severity ($\rho = 0.57$, $p < 0.01$), although richness distributions overlapped substantially among severity classes (Figure 1B).

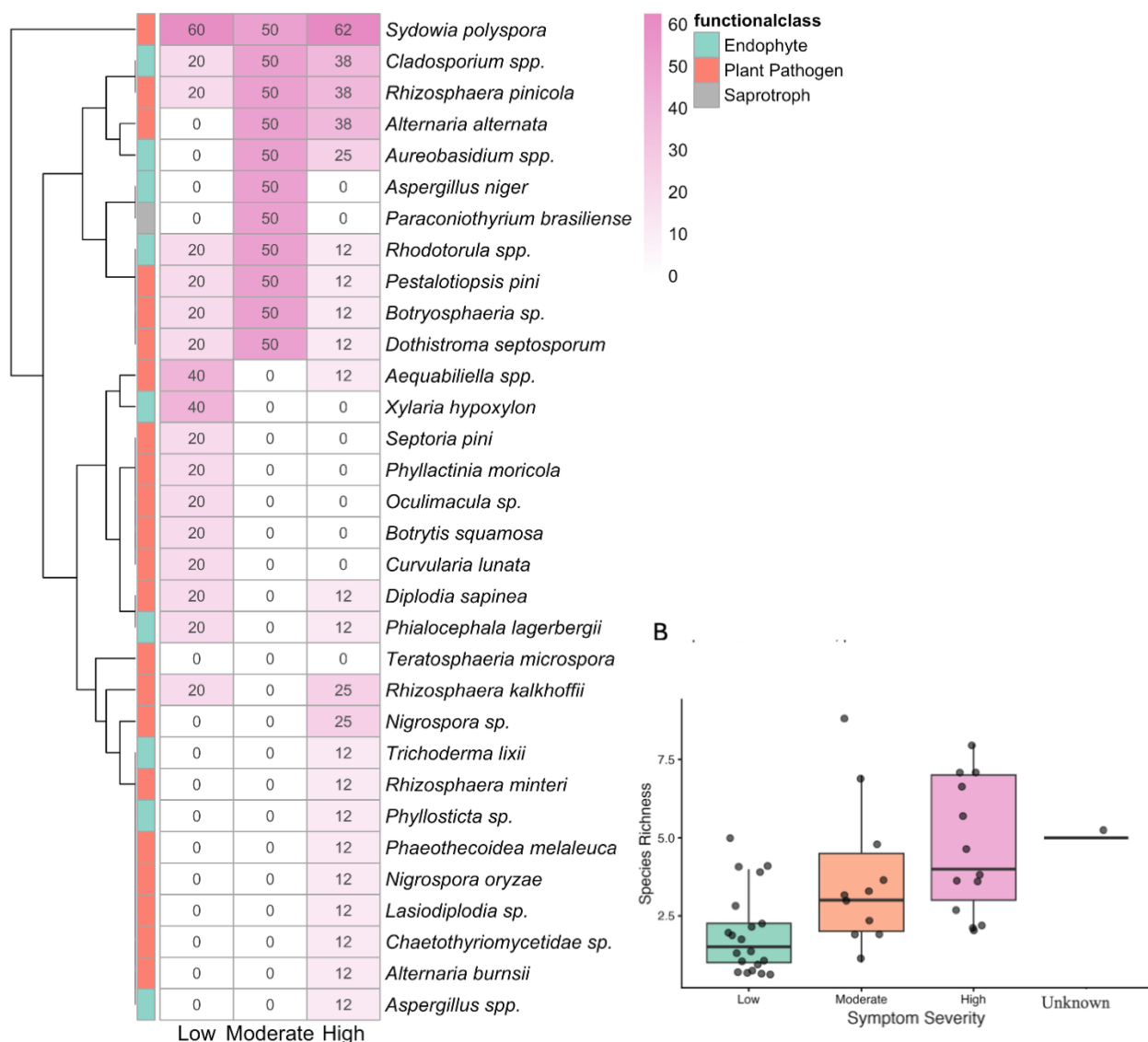


Figure 1. Fungal taxa recovered from symptomatic loblolly pine needles and species richness across symptom-severity categories. A. Prevalence heatmap of fungal taxa recovered from low-, moderate-, and high-symptom-severity trees. Values indicate the percentage of sampled trees within each severity category from which each taxon was recovered. Rows are hierarchically clustered according to similarity in prevalence profiles. Cell color intensity and overlaid values indicated the percentage of isolates in which each taxon was detected within each severity category. The row annotation bar indicates functional guild membership (endophyte, plant-associated taxon, or saprotroph). B. Relationship between per-tree species richness and symptom severity, showing a significant positive association. Points represent individual sampled trees; boxes show the median and interquartile range, and whiskers extend to 1.5 times the interquartile range. Species richness increased significantly with symptom severity (Spearman rho = 0.575, $p < 0.05$).

Genome assemblies were generated for *S. polyspora* isolates, with a mean total assembly length of 32.2 Mb (standard deviation SD = 10.3Mb) and mean contig N50 value of 2.55 Mb (SD =0.67 Mb). Our assemblies had an average of 2,700 contigs per genome isolate (SD = 2,480) and GC content mean was 49% (SD = 1.7%). Because there were some isolates that could not be adequately sequenced to a high quality due to insufficient DNA quality, we used BUSCO completeness as a filter and only those with more than 50% BUSCOs were considered for genomic analyses (filtered N=25; Supplementary Figure 3A). The *S. polyspora* isolate with highest BUSCOs completeness was selected as the reference genome for population analyses. This assembly had 172 scaffolds and 220 contigs totaling 27.96 Mb in length and contained only 0.20% gaps. Scaffold and contig N50 values were 628 kb and 421 kb, respectively. BUSCO analysis against 3,786 conserved genes showed that 96.5% were complete (96.3% present as single copy and 0.2% duplicated), 0.4% were fragmented, and 3.1% were missing, with ~2.1% of complete BUSCOs containing internal stop codons. Following reference-based mapping across all *S. polyspora* isolates, we identified 2,241,172 SNPs, and 5,670 5-kb windows contained at least one SNP, with an average of 395.26 SNPs per SNP-containing window.

PCA of LD-pruned genome-wide SNPs revealed substantial genomic variation among *S. polyspora* isolates but no discrete clustering by geographic region or symptom-severity class. PCA explained 48.69% of the total variation we observed among isolates (PC1 =39.6%; PC2 =9.09%), but isolates from different regions and symptom-severity classes overlapped broadly in ordination space (Figure 2). Contrasts within the North and between North High and South High had mean F_{ST} values close to zero, indicating little to no differentiation among those groups. In contrast, the South High vs. South Low/Moderate comparison had a strong signal

of structure (mean F_{ST} = 0.37; Supplementary Figure 4), and the South vs. North regional contrast exhibited modest but consistent differentiation (mean F_{ST} = 0.11). Comparisons involving the samples outside of Alabama had means near zero, again consistent with weak structure. Thus, although genome-wide differentiation in *S. polyspora* was elevated between severity groups in the South, severity-associated population structure was not consistent across regions.

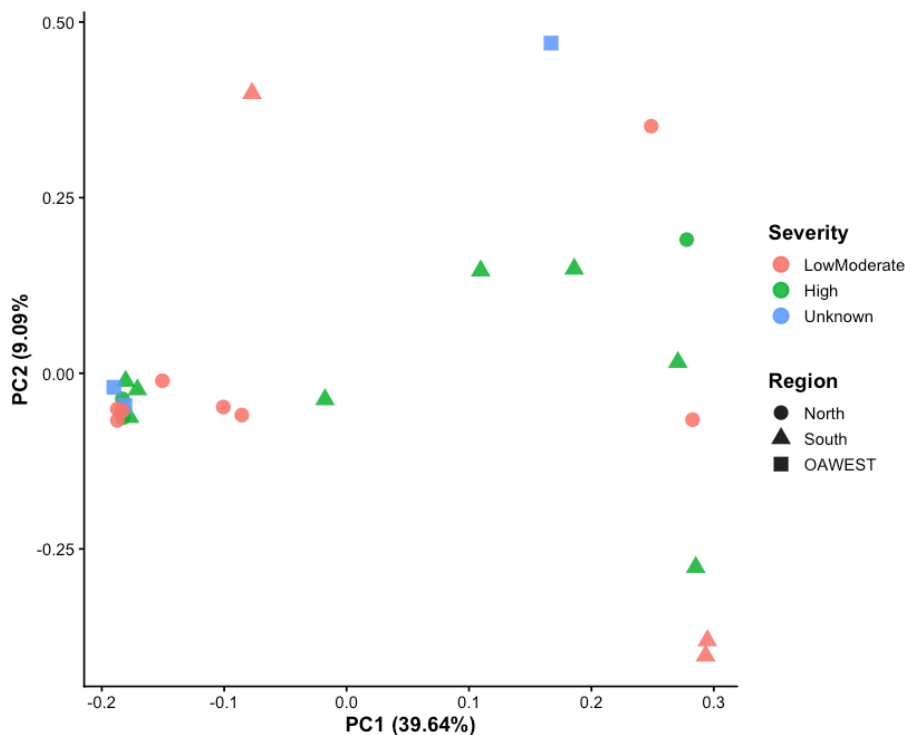


Figure 2. Principal component analysis (PCA) of *S. polyspora* isolates based on LD-pruned genome-wide SNPs. The first two principal components explained 39.64% and 9.09% of the total genomic variation, respectively. Points are colored by symptom severity and shaped by geographic region. The isolates showed substantial overlap across severity and regional groups, with no clear separation by either factor.

Window-based analyses revealed substantial heterogeneity in the genomic distribution of differentiation. We assessed population structure by estimating pairwise genome-wide F_{ST} in 5-kb windows among the BSNB symptom severity category, grouped per region (North High;

North Low/Moderate; South High; South Low/moderate; Figure 3B) as well as region independently (North, South and OA; Figure 3B). Across these 5-kb windows, F_{ST} estimates revealed differences in the genomic distribution of differentiation among comparisons (Figure 3). Most severity contrasts showed low differentiation across the genome, with F_{ST} values centered near zero and scattered windows of elevated differentiation (Figure 3A). In contrast, the South High–South Low/Moderate comparison showed broadly elevated F_{ST} across scaffolds rather than differentiation restricted to a small number of genomic regions. The North–South regional comparison similarly showed modest but broadly distributed positive differentiation, whereas comparisons involving outside-Alabama isolates were more variable and generally centered near zero (Figure 3B).

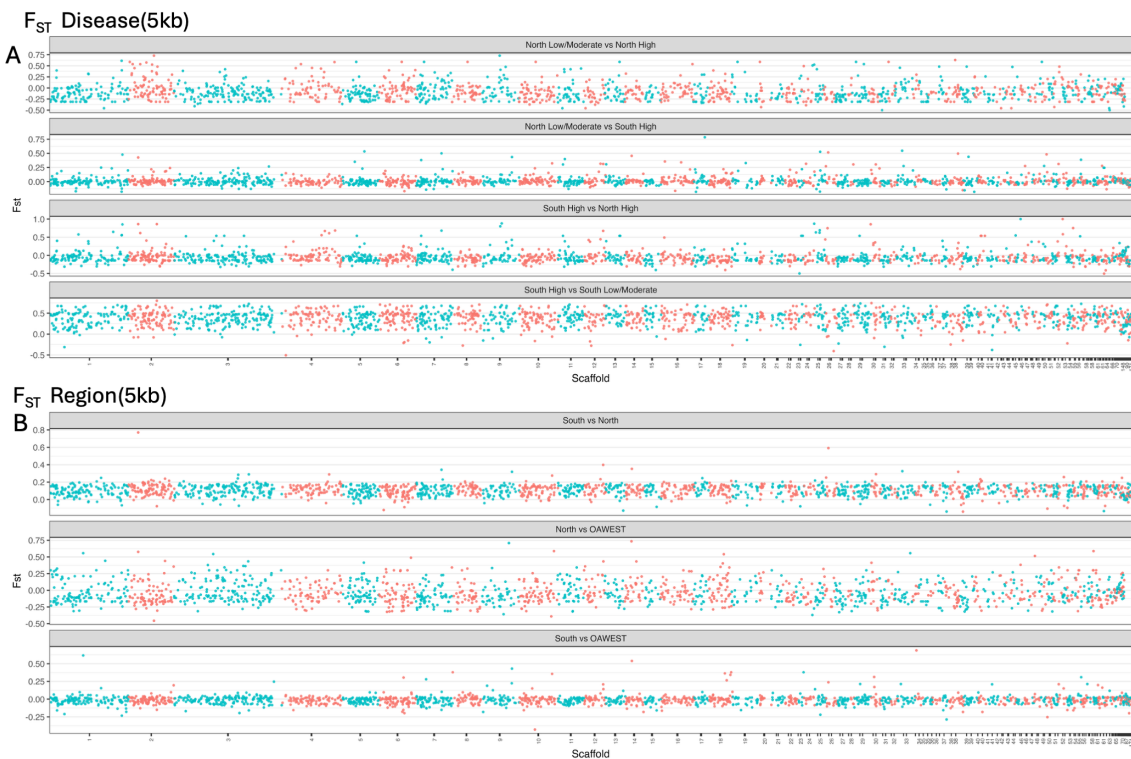


Figure 3. Patterns of differentiation of *S. polyspora*. Genome-wide F_{ST} estimated in 5-kb windows for each pairwise contrast between symptom severity (A) and regional (B) groups, illustrating mostly low differentiation with scattered windows of elevated divergence along

multiple scaffolds. Scaffolds are arranged largest to smallest along the x-axis, with alternating colors for clarity.

Genome-wide nucleotide diversity and Tajima's D varied substantially across 5-kb windows (Figure 4).. Across all isolates, we observed a mean nucleotide diversity of 8.74×10^{-3} (median = 6.75×10^{-3} , SD = 7.2×10^{-3} ; Figure 4A). Most windows clustered near the genome-wide mean however some scattered peaks occurred on multiple scaffolds. When summarized by geographic and symptom-severity groups, nucleotide-diversity distributions overlapped broadly, although the magnitude and variance of diversity differed among some groups (Figure 4C). Although southern isolates exhibited slightly elevated median diversity relative to northern isolates, severity contrasts within regions were minor relative to within-group variance. Likewise Tajima's D varied across the genome with a mean of 0.184 (Figure 4B). Tajima's D distributions similarly overlapped extensively among severity groups, with all groups exhibiting a mixture of positive and negative windows (Figure 4D). These window-based analyses indicate substantial heterogeneity in genomic variation across *S. polyspora* genomes but no consistent difference in nucleotide diversity or allele-frequency patterns associated with symptom severity.

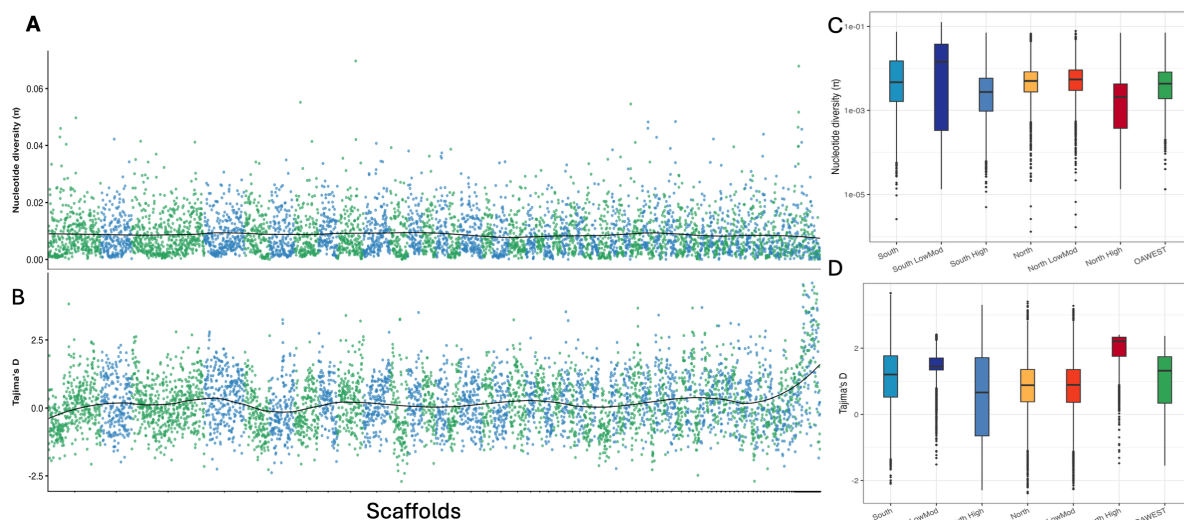


Figure 4. Genome-wide nucleotide diversity and Tajima's D for *Sydowia polyspora* estimated in 5-kb windows. A. Nucleotide diversity across windows for all population combined (disease and region groups), with scattered windows of elevated diversity but no large contiguous peaks. B. Tajima's D across windows for all populations (disease and region groups), indicating only modest differences. Scaffolds are arranged in alternating colors. C. Distribution of nucleotide diversity across regional-severity group across 5-kb windows, with mixtures of slightly negative and positive values rather than extended regions of strong deviation from neutrality. D. Distribution of Tajima's D among regional-severity groups with broad overlap in distributions

Discussion

Sydowia polyspora was consistently recovered from symptomatic *P. taeda* across the full symptom-severity gradient, extending our previous characterization of this fungus as a widespread component of symptomatic loblolly pine needles in Alabama [cite Folorunso et al., first-detection paper we can submit it as a related paper for this one]. Here, we asked whether genomic variation within this widespread fungal associate corresponded to variation in host symptom severity. Isolates from different symptom-severity classes overlapped broadly in genome-wide PCA, and differentiation between severity groups was minimal in the northern sampling region. Population-genomic studies of other pine needle pathogens provide useful context for the absence of a consistent severity-associated pattern in *S. polyspora*. Populations of *Dothistroma septosporum*, for example, show strong signatures of geographic history and introductions across their global range, while European *D. pini* populations are structured more strongly by geographic location than by host species (Barnes, Wingfield, et al., 2014). More directly relevant to brown spot needle blight, population genomic analyses of *Lecanosticta acicola* have identified two divergent lineages as well as ongoing genomic admixture where those lineages co-occur (Marcet-Houben Marina et al., 2024). Thus, although *S. polyspora* was common across symptomatic trees, our data provide little evidence for a single genetically differentiated lineage associated with greater symptom severity.

331

332 The relationship between *S. polyspora* genomic variation and symptom severity was not
333 uniform across geography. In the southern region, high-severity isolates were substantially
334 differentiated from low/moderate-severity isolates (mean $F_{ST}=0.37$), despite the absence of
335 comparable differentiation in the North. Moreover, window-based F_{ST} estimates showed that
336 the southern contrast reflected broadly elevated differentiation across the genome rather than a
337 small number of highly differentiated genomic regions. This genome-wide pattern is more
338 consistent with differentiation between genetic backgrounds than with divergence limited to a
339 small number of severity-associated loci (Flier et al., 2003; Kelly & Ward, 2018; Linde et al.,
340 2002). However, population differentiation alone cannot establish the process producing that
341 structure or its relationship to disease phenotype. Population structure in fungal pathogens can
342 reflect geographic isolation, introduction history, dispersal, recombination, and other
343 demographic processes, and these patterns need not correspond directly to pathogenic
344 phenotypes (Barnes, Wingfield, et al., 2014; Plissonneau et al., 2017). The contrasting
345 northern and southern results therefore raise the possibility that associations between *S.*
346 *polyspora* genomic background and host symptom severity are geographically contingent
347 rather than species-wide, while leaving unresolved whether the southern association reflects
348 fungal traits related to symptom expression or population structure generated by other
349 evolutionary and demographic processes.

350

351 The southern differentiation nevertheless warrants cautious interpretation because the South
352 Low/Moderate group contained only three isolates. Greater population-level replication will
353 be necessary to determine whether the observed association between genomic background and

symptom severity is reproducible or reflects population history, spatial sampling, host-associated variation, or other unmeasured factors. Importantly, even a reproducible association would not establish that fungal genotype contributes directly to symptom severity. Population-genomic approaches can reveal associations between genomic and phenotypic variation, including traits such as virulence and host specialization, but establishing those relationships requires appropriate population sampling and phenotypic characterization (Grünwald et al., 2016b). Expanded genomic sampling across regions, coupled with controlled inoculation experiments using representative isolates, could therefore test whether differentiated *S. polyspora* genomic backgrounds also differ in their effects on host symptom development. The magnitude and genome-wide distribution of differentiation observed in the South provide a clear hypothesis for such work.

Genomic variation within *S. polyspora* was also heterogeneous across the genome. Nucleotide diversity and Tajima's D varied substantially among genomic windows, with both elevated and reduced values distributed across multiple scaffolds, but neither statistic showed a consistent relationship with symptom-severity class. Such heterogeneity is increasingly recognized as an important feature of fungal genome evolution. In several filamentous plant pathogens, genomic regions differ markedly in their rates and patterns of evolution, and rapidly evolving or accessory regions can harbor variation associated with host interactions and pathogenicity (Dong et al., 2015; Frantzeskakis et al., 2018). Whether the heterogeneous patterns observed here reflect comparable genome organization in *S. polyspora* remains unknown. Our analyses were not designed to identify loci under selection or associate particular genomic regions with virulence, but they nevertheless establish considerable

genomic variation within *S. polyspora* and provide a baseline for future work examining whether particular components of that variation are associated with host interactions or other fungal phenotypes.

The genomic results also need to be considered in the ecological context in which *S. polyspora* was recovered. Rather than being restricted to the most severely symptomatic trees, *S. polyspora* occurred at similar prevalence across low-, moderate-, and high-severity classes, while the total richness of culturable fungi increased with symptom severity. Thus, greater symptom severity coincided with an increasingly species-rich culturable fungal assemblage rather than the simple appearance of *S. polyspora* in severely affected trees. Similar increases or shifts in fungal colonization with increasing tissue damage or disease severity have been reported in other plant and forest disease systems (Agan et al., 2021; Millberg et al., 2015). This pattern does not establish a causal role for fungal richness or interactions among taxa; increasingly damaged or senescing plant tissues may themselves provide opportunities for secondary colonization (Busby et al., 2016; Dastogeer et al., 2022). Moreover, culture-based isolation captures only a subset of the fungi present within host tissues and can favor taxa that grow readily under the selected culture conditions (Dissanayake et al., 2018). Our work does, however, highlight an important distinction between the occurrence of *S. polyspora* and genomic variation within it: prevalence changed little across symptom-severity classes, whereas genomic differentiation associated with severity was detected only within the southern region.

Conclusions

Our results provide an initial population-genomic characterization of *Sydowia polyspora* associated with symptomatic *Pinus teada* and reveal substantial genomic variation across sampled isolates. Although *S. polyspora* occurred at similar prevalence across the symptom-severity gradient, genomic differentiation associated with symptom severity was geographically variable. Isolates from different severity classes showed little differentiation in the northern region, whereas high-severity isolates in the southern region were substantially differentiated from low/moderate-severity isolates, with differentiation distributed broadly across the genome. Thus, our results do not support a single, species-wide genomic lineage associated with greater symptom severity, but instead suggest that relationships between *S. polyspora* genomic background and host symptom severity may be geographically contingent. Given the limited sample size within some groups, particularly southern low/moderate-severity isolates, this pattern requires validation with broader population sampling and experimental phenotyping. Together, these findings establish a genomic baseline for *S. polyspora* in southeastern pine systems and identify geographically variable genomic differentiation as an important target for determining whether fungal genetic background contributes to variation in host interactions or disease expression.

Author's Contribution

T.R.F. and J.R.W. design of the research, performance of the research, data interpretation and writing original draft. T.R.F., G.A.A.S., J.R.W and L.G.E Investigation, data collection, analysis and interpretation. R.O.O, J.R.W. and L.G.E Supervision, mentoring, project administration and resource acquisition. T.R.F, G.A.S, R.O.O, L.G.E and J.R.W writing, review and approved the final manuscript.

423

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438

439 **Conflicts of Interest**

440 The author's declare no competing interest regarding this submission

441

442 **Data and Code Availability**

443 All codes available on Github [https://github.com/Damseltemi/Brown_Spot_Project-] and
444 data available upon responsible request from corresponding author

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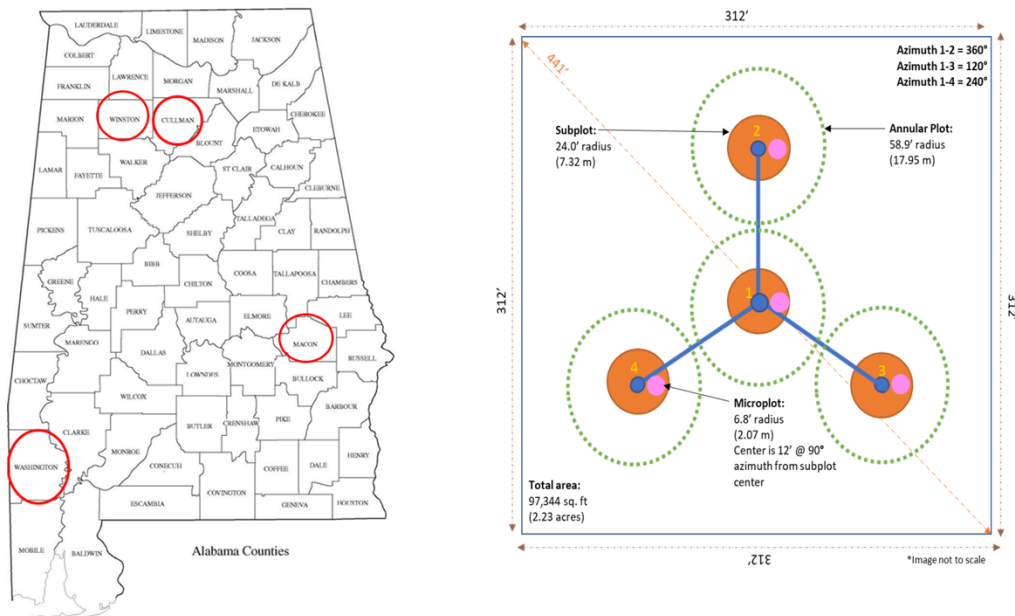
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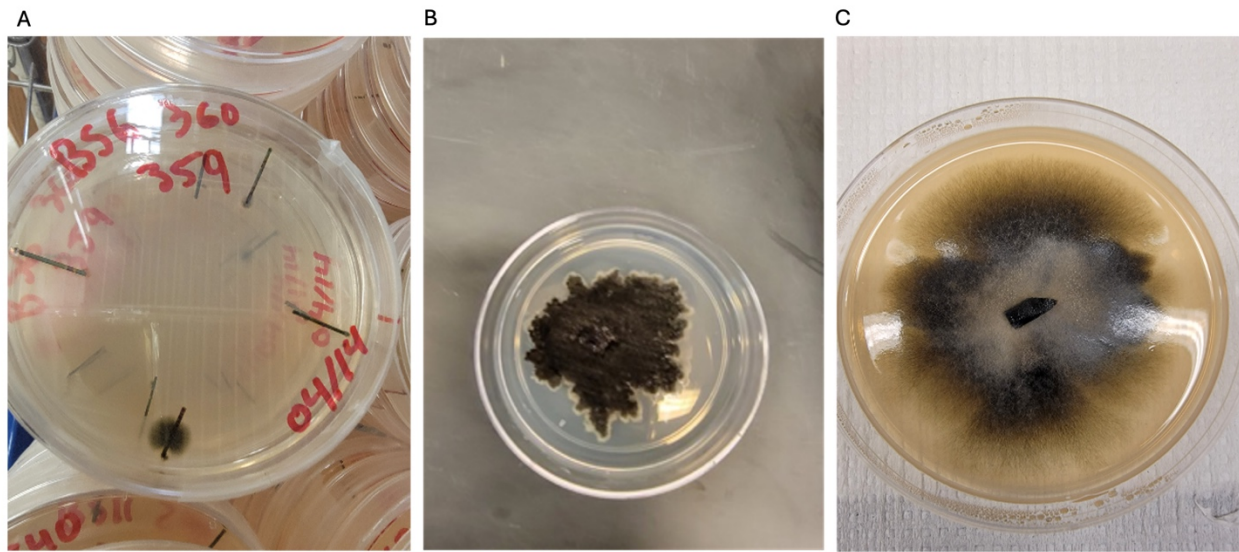
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Supplementary Figures



Supplementary Figure 1: Sampling design for Brown Spot Needle Blight surveys in Alabama (A) Map of Alabama showing the four focal counties sampled for this study: Washington County (southern region), Macon County (western region), and Cullman and Winston Counties (northern region). Across these counties, we established 22 sampling sites (9 in southern Alabama and 9 in northern Alabama and 3 in west). Base map credit: Department of Geography, College of Arts and Sciences, The University of Alabama. (B) Plot layout used within each sampling site, illustrating the central microplot and three surrounding annular subplots, each containing four focal trees. All sampling sites were surveyed monthly from March through August

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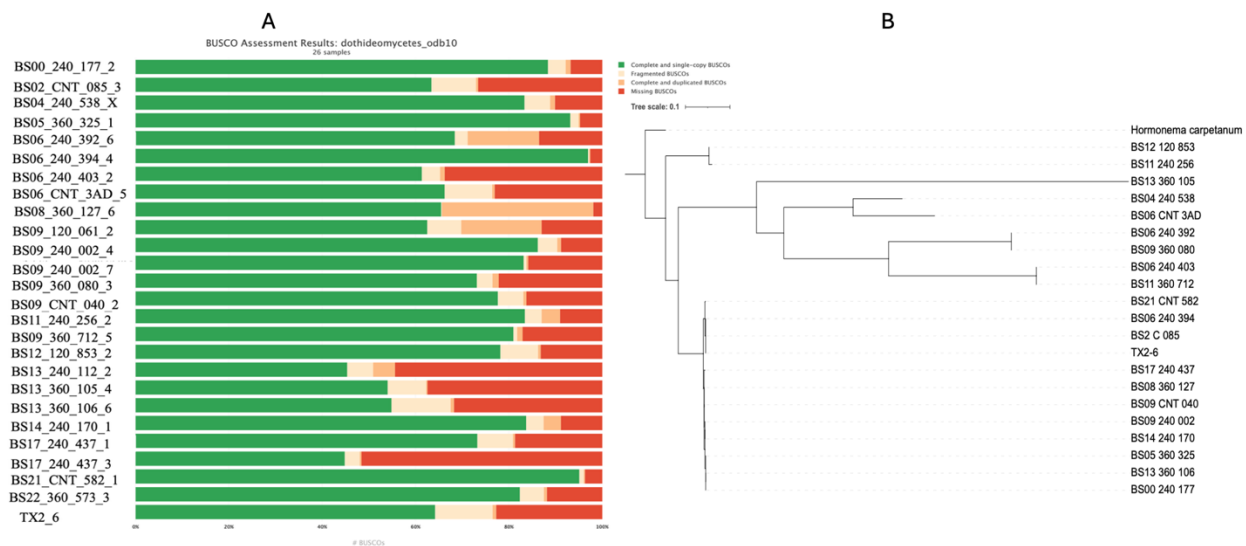
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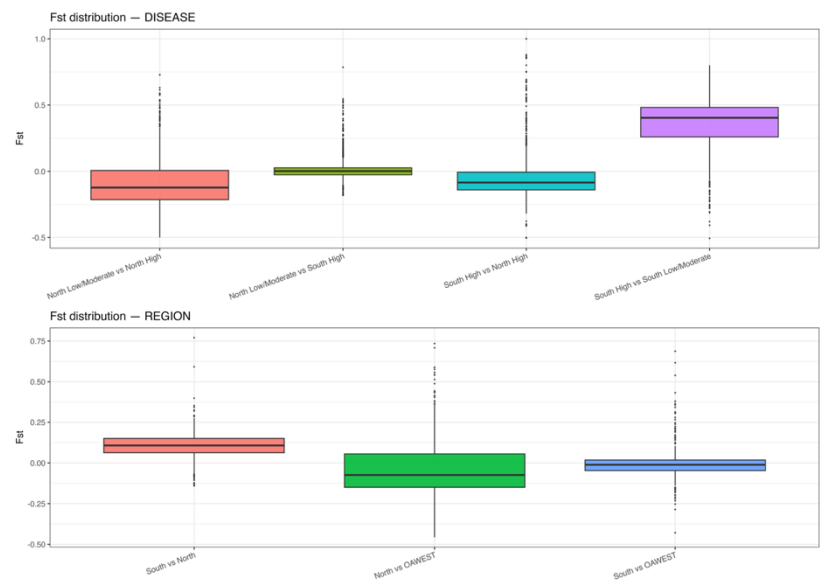
Supplementary Figure 2: Isolation of fungi from symptomatic needles. (A) Symptomatic needles washed, surface-sterilized, and plated on growth media. (B) Subcultured media used to obtain pure cultures. (C) Representative pure fungal colony derived from subculturing included in our study.

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603 **Supplementary Figure 3.** A. Genome assembly and population differentiation of *Sidowia*
604 *polyspora*. B. phylogenetic tree of our BUSCO genes, most of our isolates cluster within *S.*
605 *polyspora* species, while smaller proportion are seen to form subclades, *Hormomena carpetanum*
606 was used as an outgroup being anamorphs to *S. polyspora*. The bootstrap value and branch
607 length shows the varying length of divergences among isolates
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Supplementary Figure 4. Box distribution of window-based F_{ST} for pairwise contrasts among regional–severity groups. Across all comparisons, median F_{ST} values were close to zero, indicating generally weak genome-wide differentiation, with slightly higher and more variable F_{ST} in contrasts among northern and southern groups than within-region contrasts.