Insights into the emerging Vascular Streak Dieback disease of woody ornamentals in the United States using a metagenomic sequencing approach

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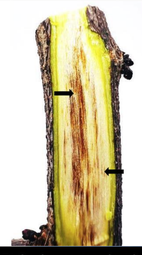
Abstract

Woody ornamentals are essential for urban landscapes and nursery production but face threats from emerging pathogens like vascular streak dieback (VSD). We used metagenomic sequencing on 106 samples from 34 woody ornamental species across seven states to identify microbial species associated with VSD. *Ceratobasidium species* (*Csp*), a fastidious fungal pathogen previously linked to VSD, was consistently detected. Genome assembly and phylogenomic analyses revealed that U.S. *Csp* isolates form a distinct genetic group closely related to *Ceratobasidium theobromae* from Southeast Asia, suggesting a recent introduction and rapid geographic expansion in the U.S. Metagenomics thus offers unmatched potential for detection and characterization of challenging fungal pathogens.

Introduction

- Ceratobasidium fungi cause vascular streak dieback (VSD) in cacao and witches' broom disease in cassava in Southeast Asia (Guest & Keane, 2007; Leiva et al., 2023).
- VSD recently emerged in woody ornamentals across the U.S., first identified in eastern redbud (Cercis canadensis) in Tennessee (2019), and now found in >12 states and 25 woody genera (Liyanapathiranage et al., 2024).
- Detection of *Ceratobasidium* sp. (*Csp*) relies primarily on ITS-based PCR assays; previous studies indicated U.S. isolates form a distinct clade closely related to *C. theobromae* (*Ct*) from Southeast Asia, possibly suggesting a novel species (Liyanapathiranage et al., 2024).

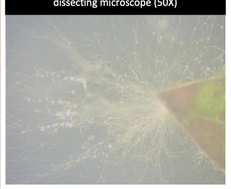
VSD-associated vascular streaking on redbud- vertical cross section view



Dieback associated with VSD

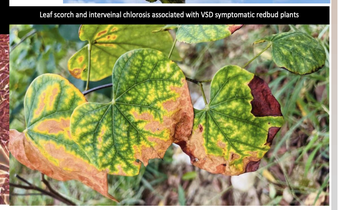
Csp growing from the incubated VSD symptomatic redbud leaf, stem and roots

Csp growth from the leaf of VSD symptomatic redbud plant as seen under a dissecting microscope (50X)



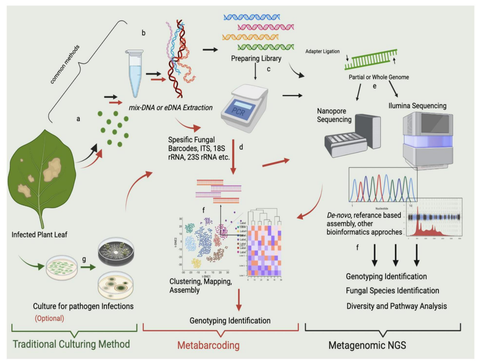
Csp growth as seen under a light microscope (600X)

Leaf scorch and interveinal chlorosis associated with VSD symptomatic redbud plants



https://www.tnstate.edu/extension/VSD%20FAct%20Sheet%20TSU_PL_Feb%2026.pdf

- *Csp* is fastidious and cannot be maintained in culture, hindering completion of Koch's postulates and genomic analyses.
- Metagenomic sequencing enables genome assembly directly from infected tissue, but is challenging because of abundant host DNA and diverse microbial backgrounds.
- We used metagenomic sequencing followed by phylogenetic and pangenomic analyses to analyze the diversity of *Csp* in the U.S. and compare it to related species.



https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1120968/full

Objectives

(i) perform a survey of bacterial, fungal, and oomycete pathogens present in symptomatic vascular tissues to determine the presence of any other pathogens besides *Csp*.
(ii) assemble *Csp* genomes from plant metagenomic and mycelium samples to elucidate evolutionary relationships among *Csp* sequences from the U.S. and with *Ct* sequences from Southeast Asia.
(iii) assess differences in gene content, including predicted virulence genes, between *Csp* and *Ct* to start unraveling potential adaptation of *Csp* to woody ornamentals and regional climate in North America.

Methods

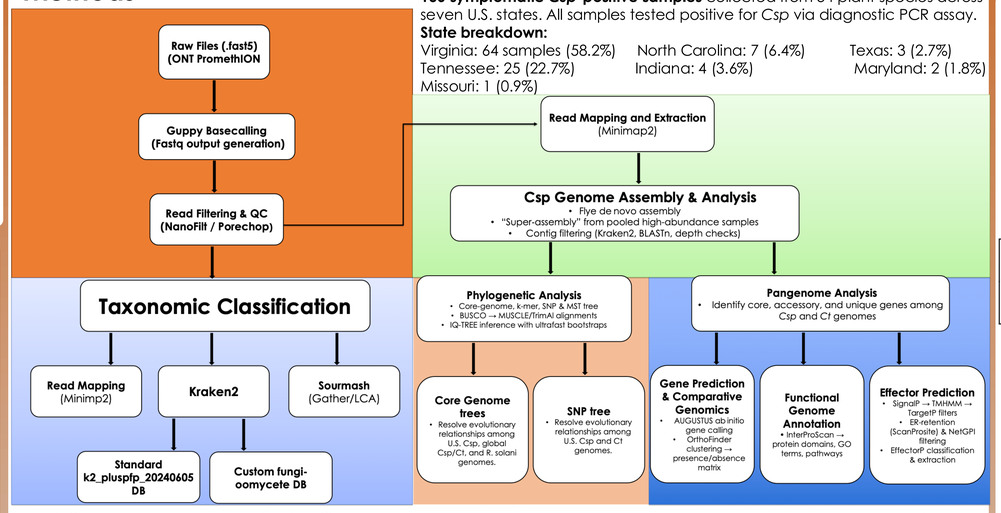


Figure 1: Overview of the metagenomic workflow, from ONT sequencing and taxonomic profiling to genome assembly, phylogenetic analysis, pangenome construction, functional annotation, and effector prediction.

106 symptomatic *Csp*-positive samples collected from 34 plant species across seven U.S. states. All samples tested positive for *Csp* via diagnostic PCR assay.
State breakdown:
Virginia: 64 samples (58.2%) North Carolina: 7 (6.4%) Texas: 3 (2.7%)
Tennessee: 25 (22.7%) Indiana: 4 (3.6%) Maryland: 2 (1.8%)
Missouri: 1 (0.9%)

Results

Deep metagenomic sequencing provides insight into microbial communities associated with VSD

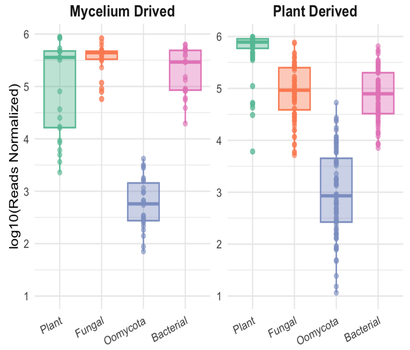


Figure 2. Boxplots of normalized read counts for plant, fungal, oomycete, and bacterial taxa in mycelium- and plant-derived samples.

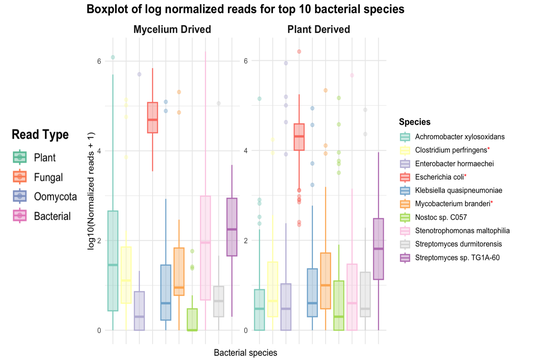


Figure 3. Boxplots of normalized reads for the top 10 bacterial species in mycelium- and plant-derived samples. Species marked * were not confirmed by additional analyses.

Genome Assemblies of *Ceratobasidium species* (*Csp*) isolates

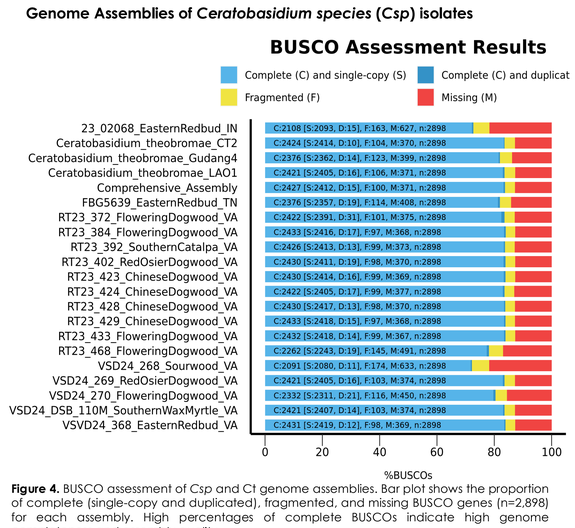


Figure 4. BUSCO assessment of *Csp* and Ct genome assemblies. Bar plot shows the proportion of complete (single-copy and duplicated), fragmented, and missing BUSCO genes (n=2,898) for each assembly. High percentages of complete BUSCOs indicate high genome completeness and assembly quality.

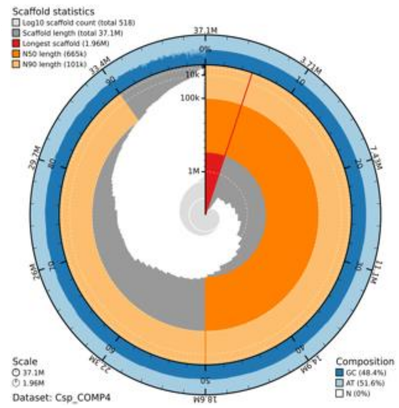


Figure 5. Snail plot of the Csp comprehensive assembly showing scaffold length distribution (total 37.1 Mb, 518 scaffolds), base composition (48.4% GC), and key assembly metrics (longest scaffold, N50, N90)

Phylogeny to resolve evolutionary relationships among U.S. *Csp* samples and related taxa.

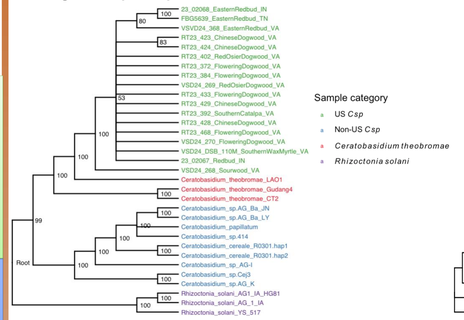


Figure 6. Core genome tree based on 76 single-copy orthologs for U.S. *Csp*, *Ceratobasidium*, and *R. solani* genomes. *R. solani* is outgroup.

Phylogeny to resolve evolutionary relationships among U.S. *Csp* isolates.

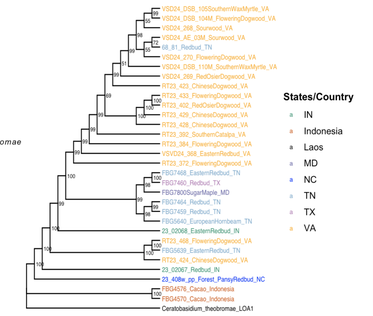


Figure 7: Maximum-likelihood SNP tree of 32 *Csp* isolates rooted on Ct LOA1, with bootstrap support at nodes.

Exploring gene content differences between genomes of U.S. *Csp* isolates and other *Ceratobasidium* and *Rhizoctonia* isolates and among U.S. *Csp* isolates

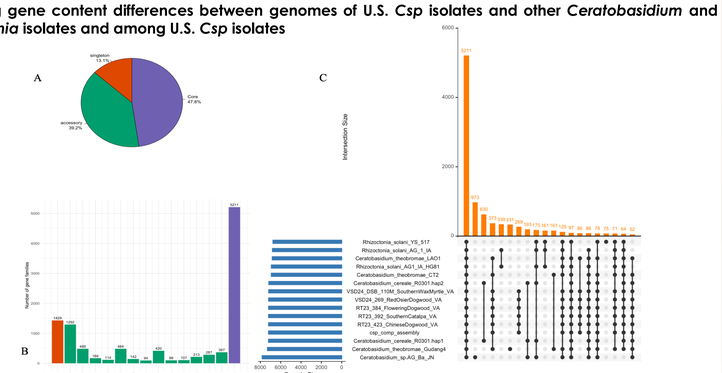


Figure 8. Pangenome analysis of *Csp*, *Ceratobasidium* and *Rhizoctonia* genomes. (A) Core, accessory, and singleton gene composition. (B) Orthogroup counts by genome. (C) UpSet plot of orthogroup intersections across genomes.

Conclusions

- Metagenomic long-read sequencing effectively enabled genomic studies of the fastidious fungal pathogen *Csp*, even in the absence of pure cultures.
- Using multiple taxonomic approaches and a custom database, we confirmed *Csp* as the sole causal agent of VSD in U.S. ornamentals, despite challenges with false positives in profiling.
- Long-read data allowed assembly of several high-quality *Csp* draft genomes, providing new insights into relationships among U.S. and related lineages and enabling a robust pangenome analysis.
- The relatively high ONT error rate limited SNP-based phylogenetic resolution, highlighting the need for continued improvements in sequencing accuracy for population-level studies.

Future Directions:

- Expand metagenomic surveys across a broader range of hosts and regions to track VSD emergence and spread.
- Integrate hybrid sequencing to improve assembly accuracy and SNP-based phylogenomics.
- Pursue functional genomics and pathogenicity studies to pinpoint genes and mechanisms underlying host specificity and virulence.
- Develop rapid molecular diagnostics based on unique markers for early detection and management.

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