Native Plant Society Report
Annika Fockler and Riley Hickman
8/8/2024

Endophytes are microbial fungi that live in the tissues of plants, typically they are beneficial to the plant but some can be pathogenic. The goal of this project was to create a profile of the endophytes currently affecting native plant species in the Blue Ridge Parkway and the Great Smoky Mountains National Park.

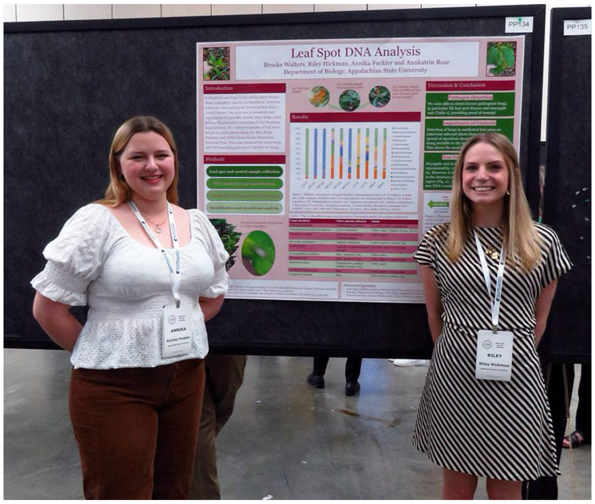
Within the vicinity of the Blue Ridge Parkway and the Great Smoky Mountains National Park, approximately 70 samples of native leafspot endophytes and their controls were collected for DNA extraction and sequencing. DNA was extracted from 67 samples and quantified, and those that possessed enough pure DNA to be processed were sent off to MR DNA Lab in Shallowater, Texas. The DNA we sent off included samples from Turk's Cap Lily (*Lilium superbum*), Mayapples (*Podophyllum peltatum*), Canadian Wood Nettle (*Laportea canadensis*), and White Snakeroot *(Nabalus altissimum*). The raw DNA sequences from these were used to find regions of similarity to other DNA sequences in the database to analyze the endophyte profile in the plants.

We used the top 10 samples with the most sequences and their controls to create a bar graph and display the endophyte profile. We decided to use fungal families to categorize the endophytes, and our findings from this grouping method saw a significant presence of two fungal families which host detrimental leafspots. The first was the fungi that causes Mayapple rust, a parasitic pathogen. The other prominent fungus was the family which includes Lily leafspot disease, a pathogen that infects the native Turk's Cap Lily, which can result in rot and later death.

The harvesting procedure of the leafspots also brought on new information regarding the spread of leafspots within a plant system. A sample of Mayapple rust was taken from an area of the plant showing the outward symptoms of bright orange cups, while its accompanying control was taken from the area of the same plant without any noticeable symptoms of leafspot infection. However, after the DNA was sequenced, the data showed that the control contained the same pathogenic fungi that affected the known infected sample. This demonstrated that controls should be taken from a separate plant and has raised more questions about the spread of leafspot endophytes within the plant. Our next question was about reinfection- do the endophytes stay in the root system, create a reservoir, and then cause reinfection when the plants come up again in the spring?

This project collected valuable information about the endophytes affecting native plant species along the Blue Ridge Parkway and Great Smoky Mountains and has branched into two projects furthering the research- one project will highlighting the effects and spread of mayapple rust and another studying lily leaf spot disease. Our project was presented on a poster at the 85th Annual Meeting of the Association of Southeastern Biologists in Chattanooga, Tennessee and at Appalachian State's Biology Research Day, where it won the People's Choice Award.

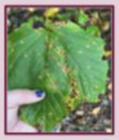
Annika and Riley collecting samples along the Porters Creek Trail in the Great Smoky Mountains, TN.



Riley and Annika presenting at the 85th Annual Meeting of the Association of Southeastern Biologists in Chattanooga, TN.

Leaf Spot DNA Analysis

Brooke Walters, Riley Hickman, Annika Fockler and Annkatrin Rose
Department of Biology, Appalachian State University



Introduction

Endophytes are fungi living within plant tissues. Many endophyte species are beneficial; however, some can turn pathogenic, lowering their host's overall fitness. Our goal was to inventory and understand the possible threats these fungi could pose to the protected ecosystems in the Southern Appalachians. We collected samples of leaf spots found on native plants along the Blue Ridge Parkway and within Great Smoky Mountains National Park. DNA was extracted for sequencing with ITS barcoding primers to identify the fungi.

Methods

Leaf spot and control sample collection

DNA extraction and quantification

ITS fungal DNA sequencing (Illumina)

Identification and mycobiome analysis

Pseudocercosporella inconspicua on *Lilium superbum*.

S2: Leaf spot sample

C2: Control sample from separate plant

C3: Control sample from unaffected leaf area on same plant as spot sample

Results

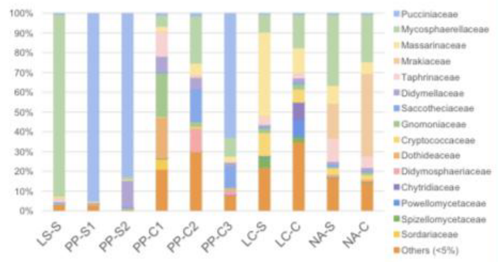


Figure 1: Relative abundance of fungal families representing at least 5% of reads in at least one of the samples. Families with lower abundances are summarized in Others. LS, *Lilium superbum*; PP, *Podophyllum peltatum*; LC, *Laportea canadensis*; NA, *Nabalus altissimum*; S, leaf spots; C, controls. Sampling locations: 1, Great Smoky Mountains National Park (GRSM); 2 & 3, ASU Dark Sky Observatory; all others, Blue Ridge Parkway (BLRI).

Table 1: Top 10 identified fungal taxa by relative abundance across all samples sequenced.

Fungi identified	Plant species affected	Notes
Allodus podophylli	*Podophyllum peltatum*	no 100% match in database
Pseudocercosporella inconspicua	*Lilium superbum*	100% match (Ingram & Levy, 2019)
Ramularia vizellae	*Laportea canadensis* (control sample)	100% match
Itersonilia perplexans	*Nabalus altissimus*	100% match to various spp.
Leotiomycete	*Laportea canadensis* (spot sample)	no 100% match in database
Aureobasidium pullulans	Misc. (present in all)	100% match
Ramularia spp.	Misc. (present in all)	100% match to various spp.
Boeremia exigua	*Podophyllum peltatum* (GRSM sample only)	100% match
Uncultured Ascomycota	Misc.	100% match to various spp.
Endophyte isolates	Misc.	100% match to various spp.

Discussion & Conclusion

Pathogen Detection

We were able to detect known pathogenic fungi, in particular lily leaf spot disease and mayapple rust (Table 1), providing proof of concept.

Importance of Controls

Detection of fungi in unaffected leaf areas on otherwise infected plants demonstrated the spread of mycelium throughout samples despite being invisible to the naked eye (Fig. 1, PP-C3). This shows the need for uninfected controls.

New Fungal Sequences

Mayapple rust in the Pucciniaceae family was represented by >70% of the DNA in PP-S1 & PP-S2. However, it did not have a matching sequence in the database due to vouchers missing the ITS-1 region (Fig. 2). Thus, this project will contribute new DNA vouchers for fungal identification.

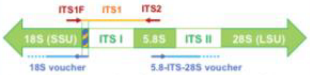


Figure 2: Placement of fungal ITS primers (red) and ITS1 reads (orange) on the rDNA locus (green) in relation to *Allodus podophylli* voucher sequences (blue, Minnis et al., 2012). Only 15 nucleotides at the end of the 18S voucher overlapped with our *A. podophylli* ITS1 sequence.

Leaf spots on *L. canadensis* (left) *and N. altissimus* (right).

References:

- Ingram & Levy (2019). Identity and symptomatology of a newly described lily leaf spot disease (*Pseudocerosporella inconspicua*) of Gray's lily (*Lilium grayi*). Can. J. Plant Pathol. 42:4, 499-507.
- Minnus et al. (2012). Taxonomy of mayapple rust: the genus *Allodus* resurrected. Mycologia 104:4, 942-950.

Acknowledgments:

- Paul Super (GRSM) and Evan Raskin (BLRI) for assistance with sample collection, and Ben Crawford for help with DNA extractions.
- Funding: Department of Biology, SAFE Grant, Appalachian State University; Tom and Bruce Shian Fund, NC Native Plant Society.
- Research permits: National Parks Service, Great Smoky Mountains National Park GRSM-02201, Blue Ridge Parkway BLRI-00528.