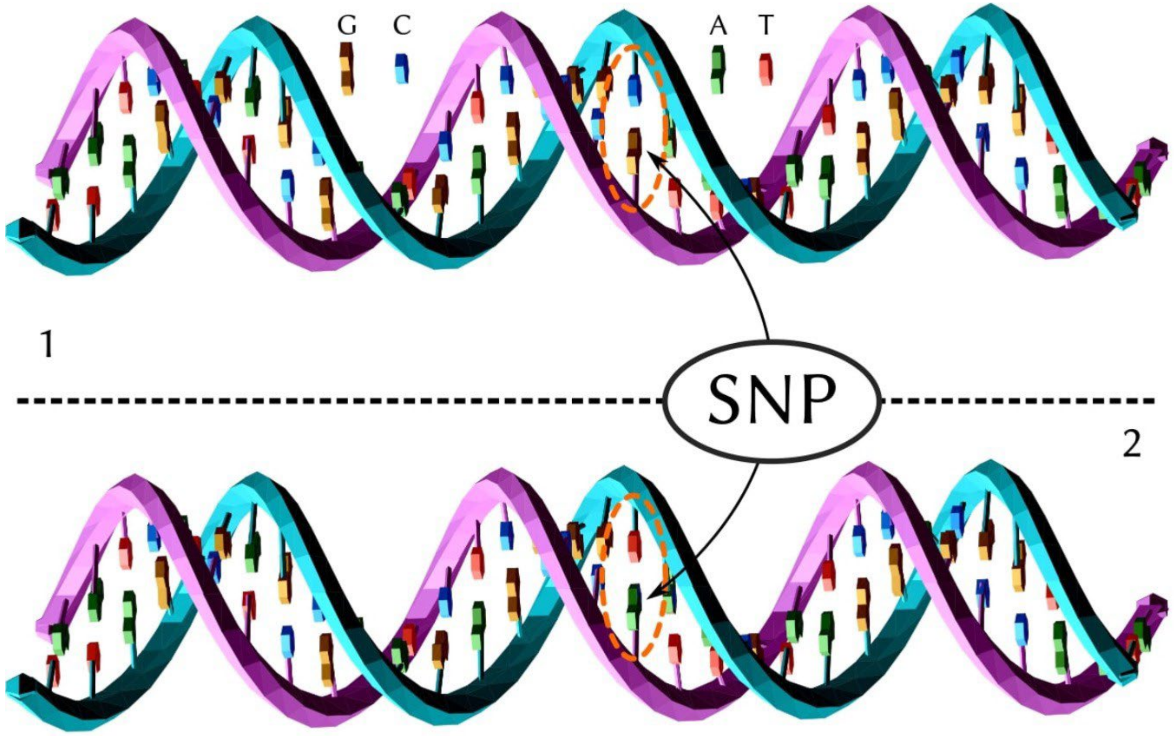
Using genetic variants and evolutionary history of the fungal pathogen *Lecanosticta acicola* to understand needle blight



Temitope Ruth Folorunso

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willoughby
conservation and genomics lab

1. Introduction

- Forests ecosystem are constantly under threat due to shifting environmental conditions and biological invaders such as plant pathogenic fungi
  - *Dothistroma septosporum,*
  - *Lophodermella sulcigena,*
  - *Lecanosticta acicola e.t.c*

- A disturbance to this forests can be catastrophic

- Mitigating economic risks could be accomplished by limiting some of these stressors, including those related to plant pathogenic fungi

Brown spot needle blight disease

Caused by ***Lecanosticta acicola***

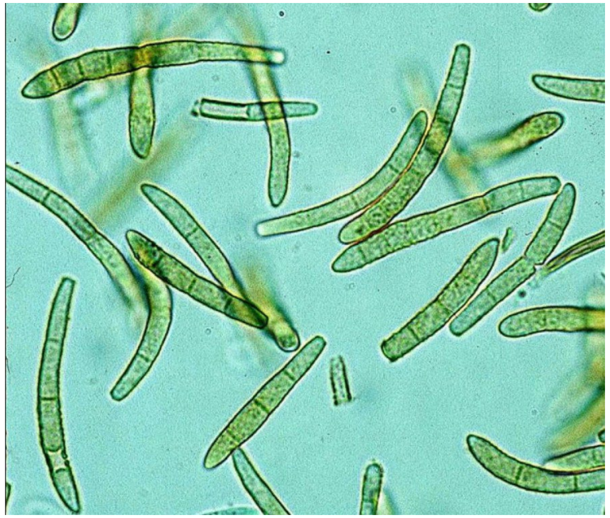


Photo: EPPO Global Database

Photo: T.F, Auburn Uni

- Yellow-brown spot
- Necrosis of needle tips
- Total defoliation of the tree

Photo: T.F, Auburn Uni

Life Cycle of *Lecanosticta acicola*

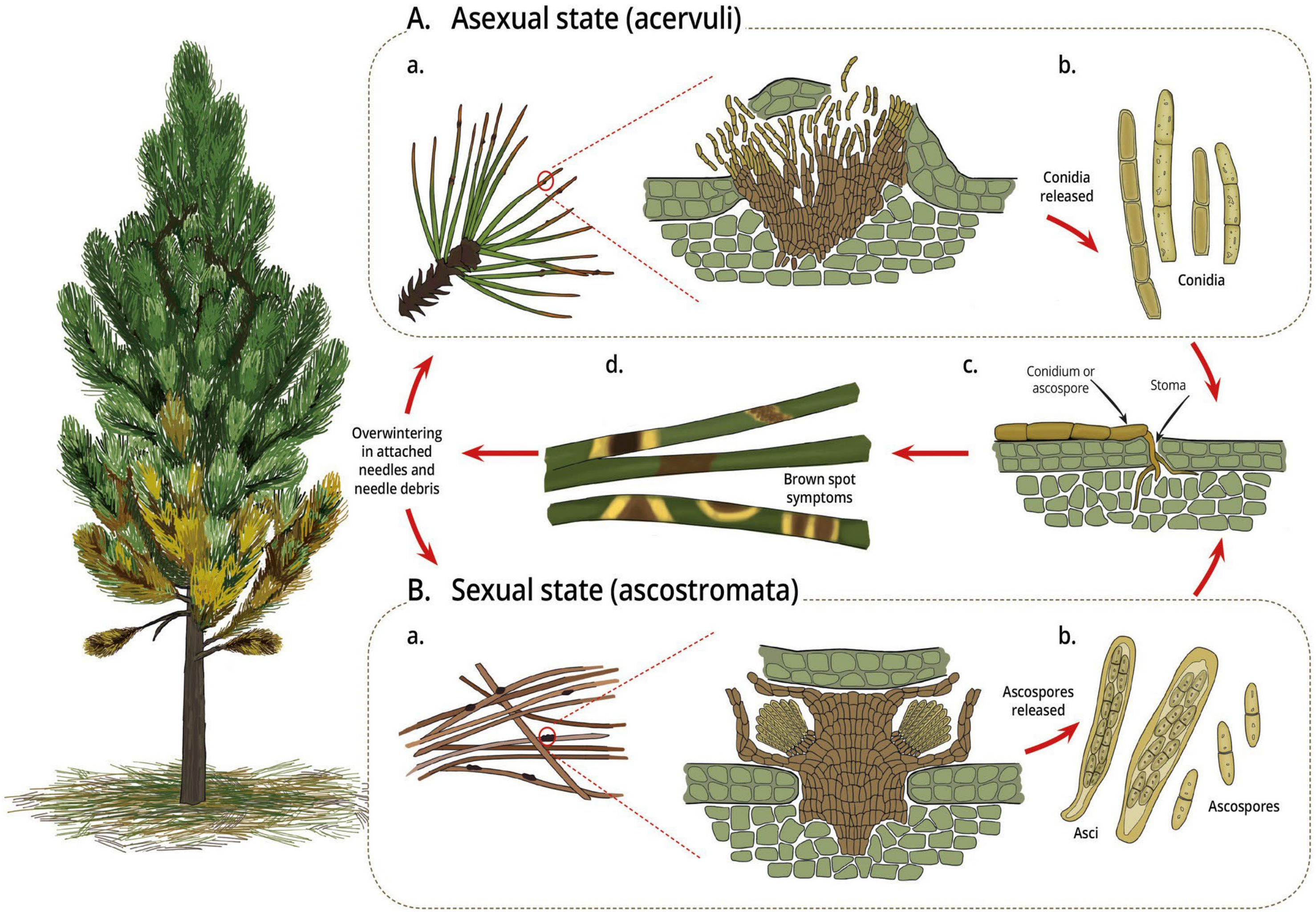


Photo: Vander Nest et al., 2019

Asexual stage: *Lecanosticta acicola* develops acervuli on attached needles and needle debris, releasing conidia that infect new season needles through stomata, causing brown spot symptoms.

Sexual stage: Ascostromata develop on dead needles from previous infections, releasing ascospores in spring that infect new season needles through stomata, also leading to brown spot symptoms.

History of *L. acicola* and brown spot needle blight

Known infections

P. palustris - Late 1800’s

P. taeda

Almost 50 pine species globally

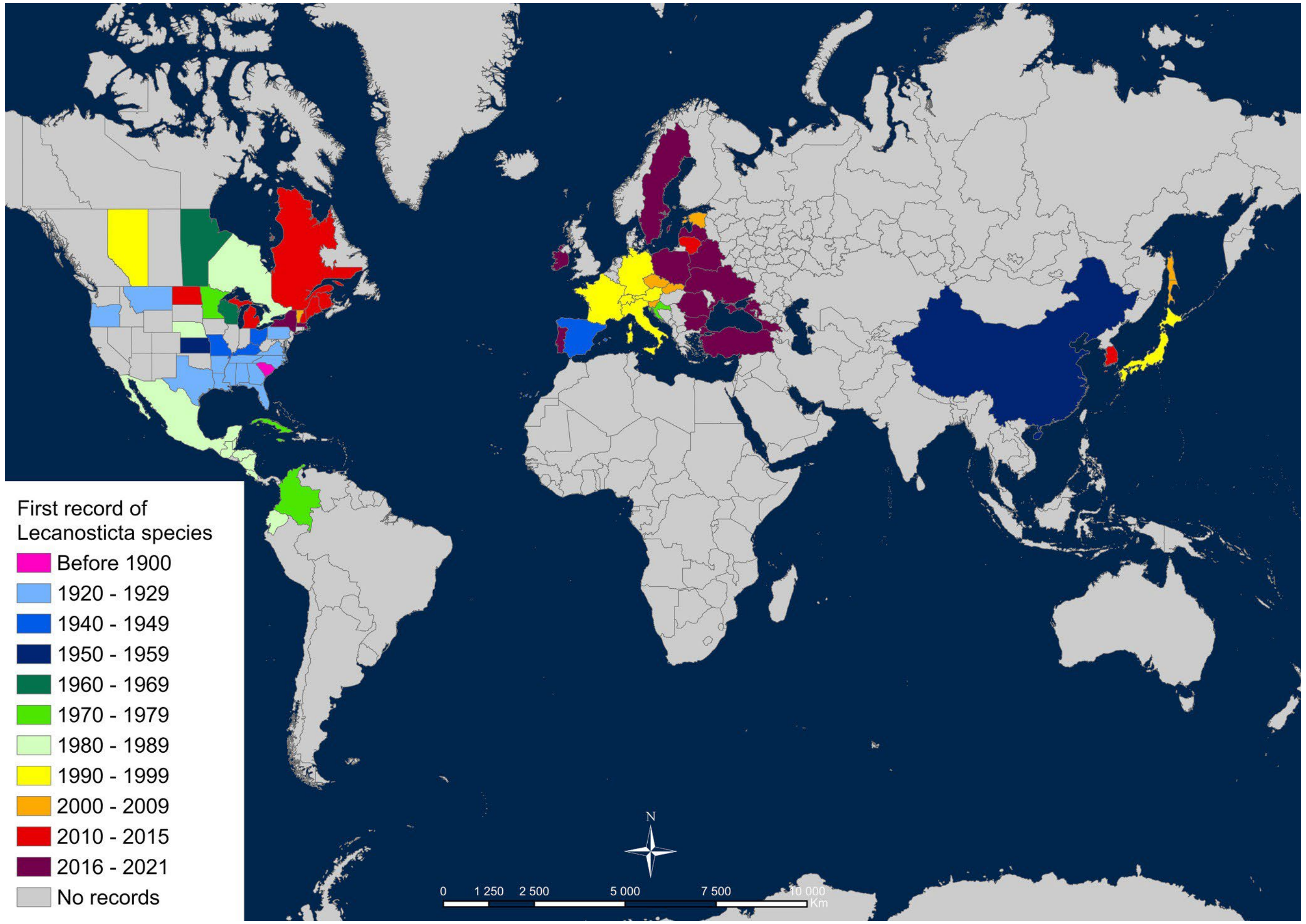
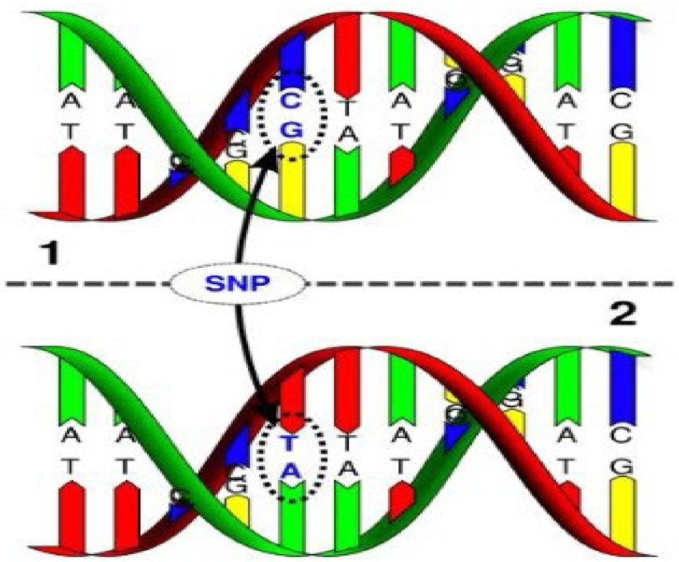


Photo: Tubby et al., 2023

Genetic diversity studies of *L. acicola*



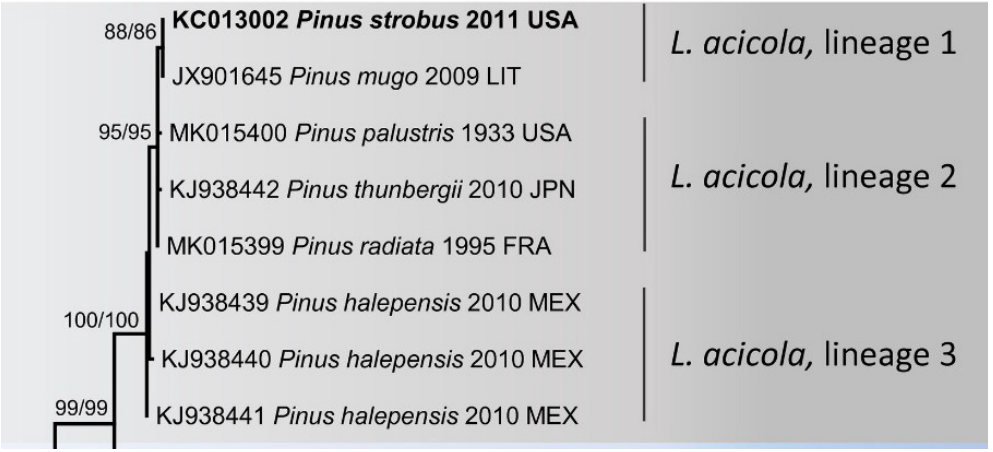
- Changes in the allele, genes and nucleotide

- Heritable variation within and between populations of organisms

Genetic diversity of the pine pathogen *Lecanosticta acicola* in Slovenia and Croatia

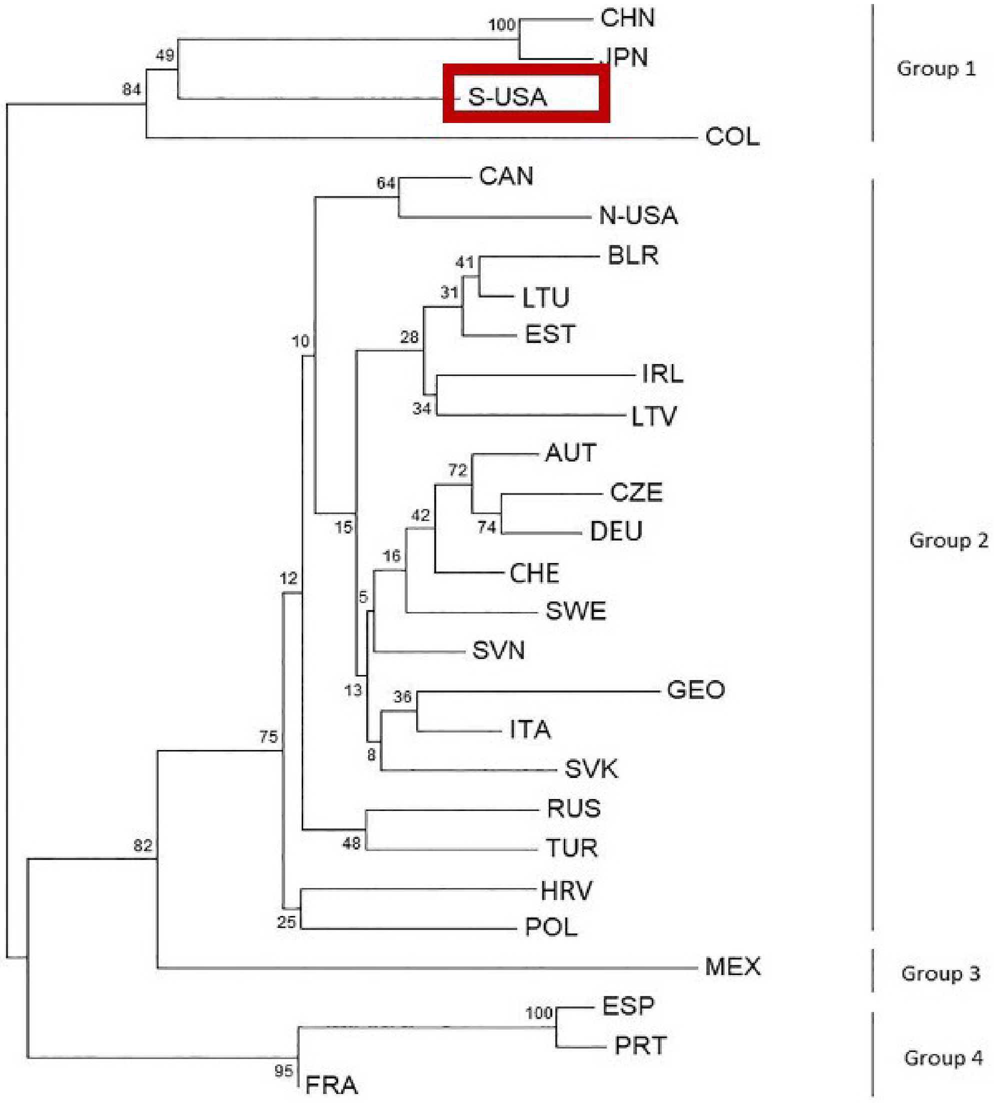
D. Sadiković, B. Piškur, I. Barnes, T. Hauptman, D. Diminić, M. J. Wingfield, D. Jurc

First published: 18 March 2019 | **https://doi.org/10.1111/ppa.13017** | Citations: 11



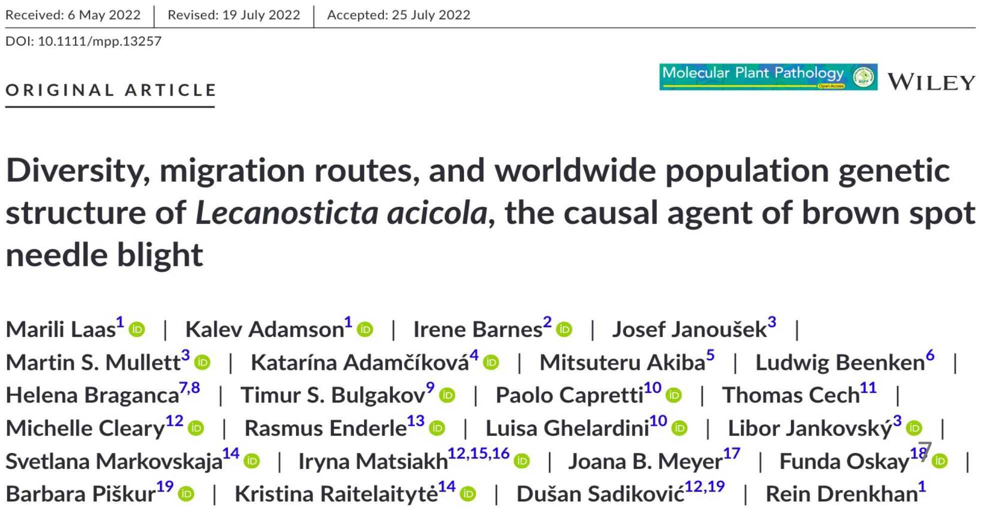
Genetic diversity studies of *L. acicola*

w i l l o u g h b y
conservation and genomics lab



Understand how pathogens evolve over time and adapt to different environments and hosts

- Measure the genetic variability of *L. acicola* in all sampled isolates
- Characterize population structure to understand the distribution of variants and pathways for infection of loblolly stands


Received: 6 May 2022 | Revised: 19 July 2022 | Accepted: 25 July 2022
DOI: 10.1111/mpp.13257

ORIGINAL ARTICLE

Molecular Plant Pathology WILEY

Diversity, migration routes, and worldwide population genetic structure of *Lecanosticta acicola*, the causal agent of brown spot needle blight

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Objectives

1. Use genetic sequencing to understand *L. acicola* evolution
    - Measure the genetic variability of *L. acicola* in all sampled locations and characterize population structure to understand the distribution of variants and pathways for infection of loblolly stands

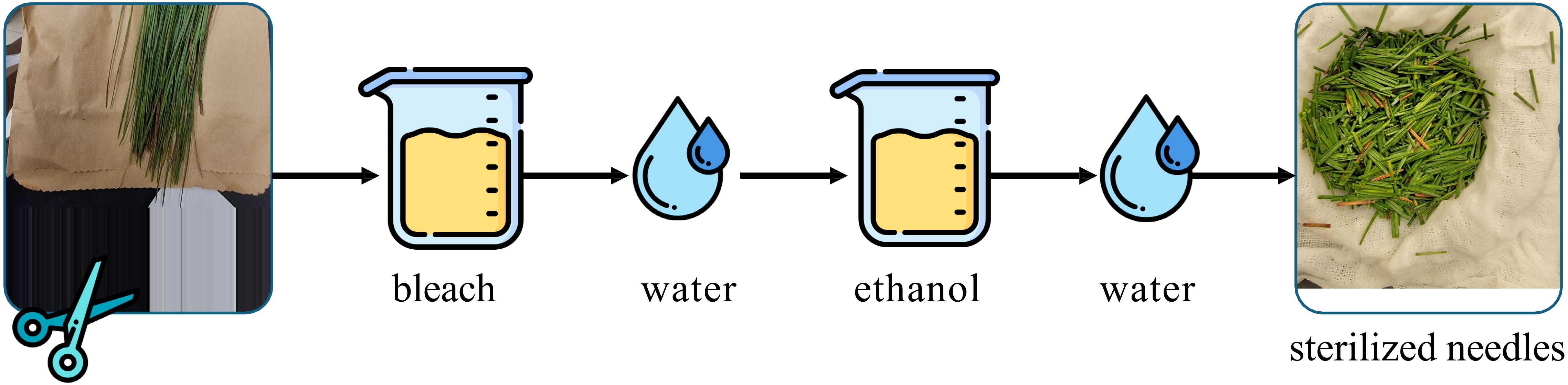
2. Quantify movement of fungal lineages between our identified populations and use these patterns to make predictions about the dominant method for *L. acicola* spread at these spatial scales

Methods

Collecting symptomatic needles from diseased trees

Photo: Graduate Students, Auburn

Needle sterilization process



bleach → water → ethanol → water → sterilized needles

Fungal cultivation and isolation

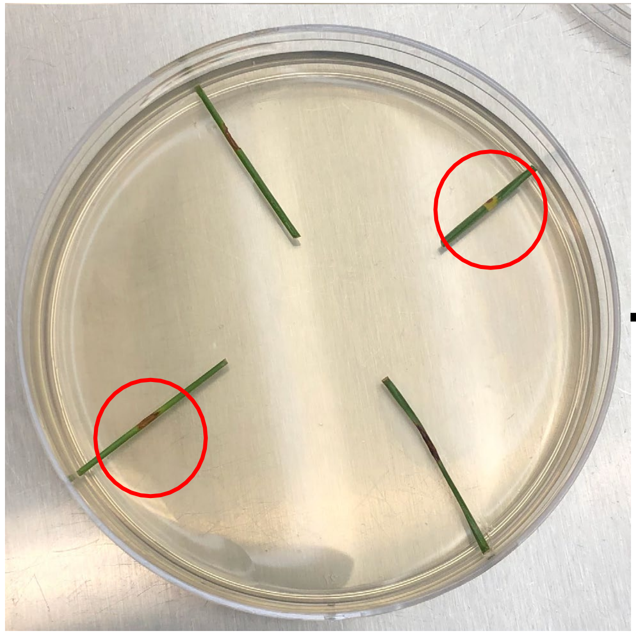
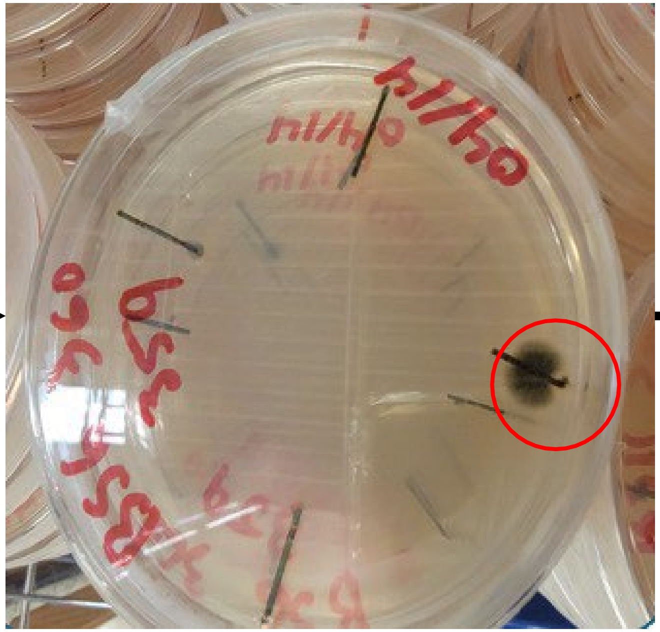


Plate needle samples that have spots

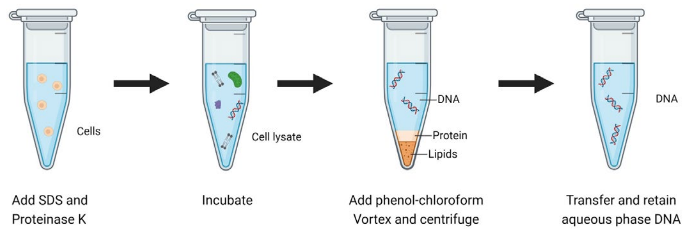


Subculture growth with suggestive morphology into same media

Pure fungus isolate

Photo: Gabriel, Temitope, Auburn

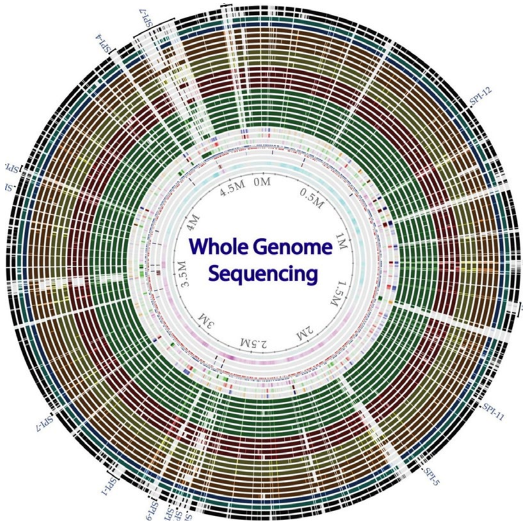
DNA extraction, quantification and confirmation



Extraction – phenol chloroform and EZNA Plant & Fungal Kit

Quantification – Qubit, nanodrop, electrophoresis

Whole genome sequencing of *L. acicola*



Create sequencing libraries, one for each confirmed *L. acicola* isolate

Sequence each libraries using Oxford Nanopore MinIon sequencer

Assemble and analyze genome sequences

Preliminary results

Detection and identification of *L. acicola*

- 52 total sites out of sampled
- 47 from loblolly trees
- 5 from long-leaf trees

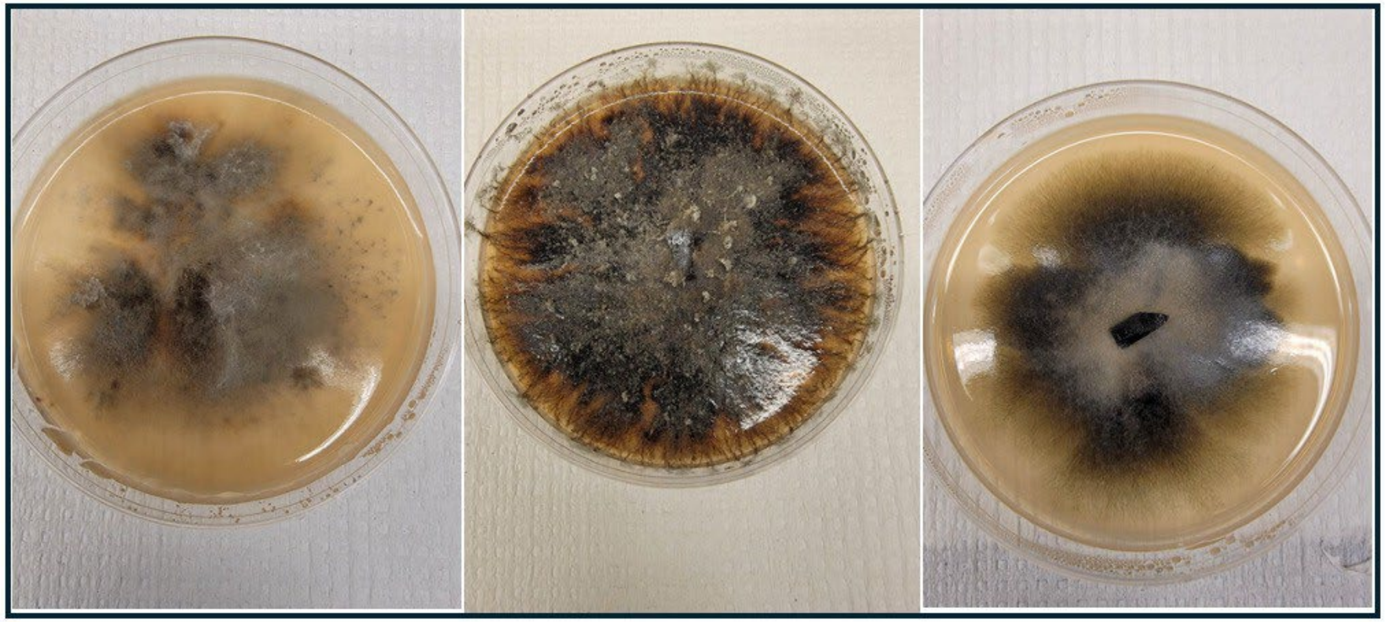
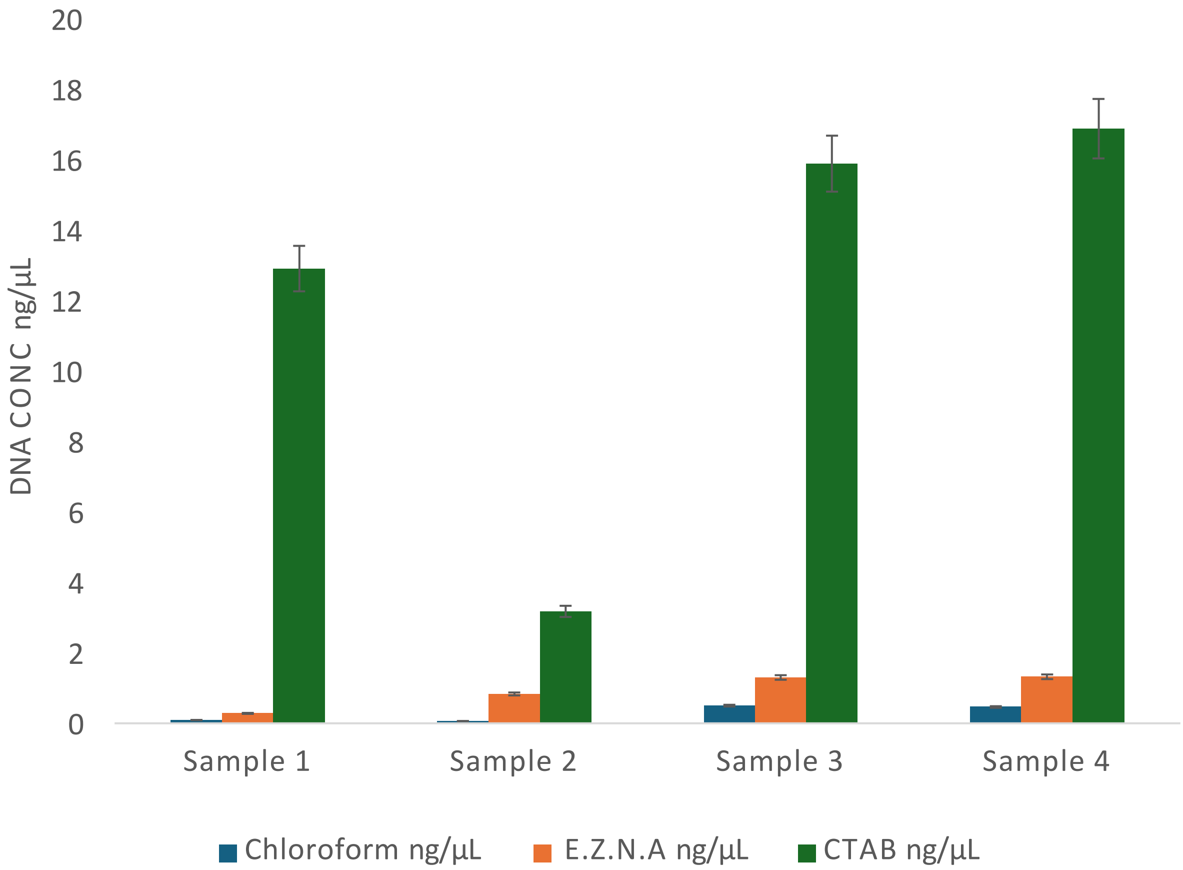


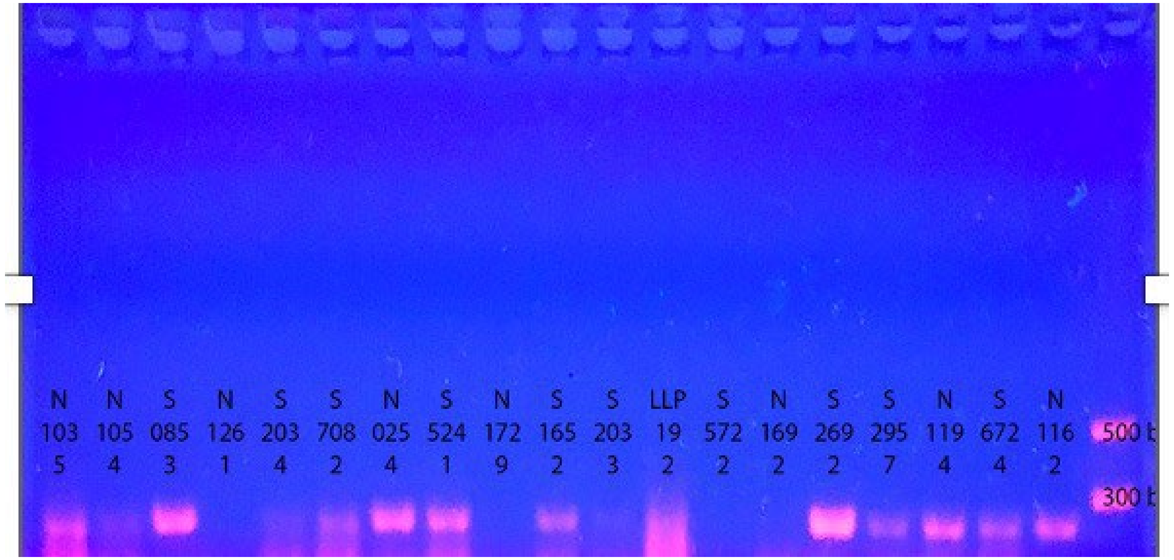
Photo: Gabriel and Temitope, Auburn Uni

Preliminary results



DNA concentration of isolates using three different protocol.

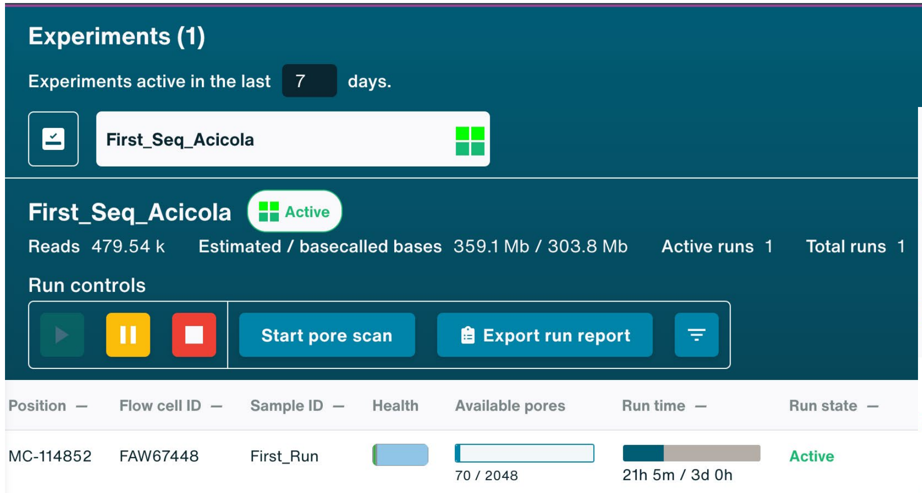
LAtef Primers (Loos, 2011) - TEF1



Gel electrophoresis image confirming *L. acicola* isolate via PCR separated on 1% agarose gel.

Preliminary results MinION ouput

After struggling to get high molecular weight DNA (required liquid media!), we got first sequenced data



Experiments (1)

Experiments active in the last 7 days.

Search experiments

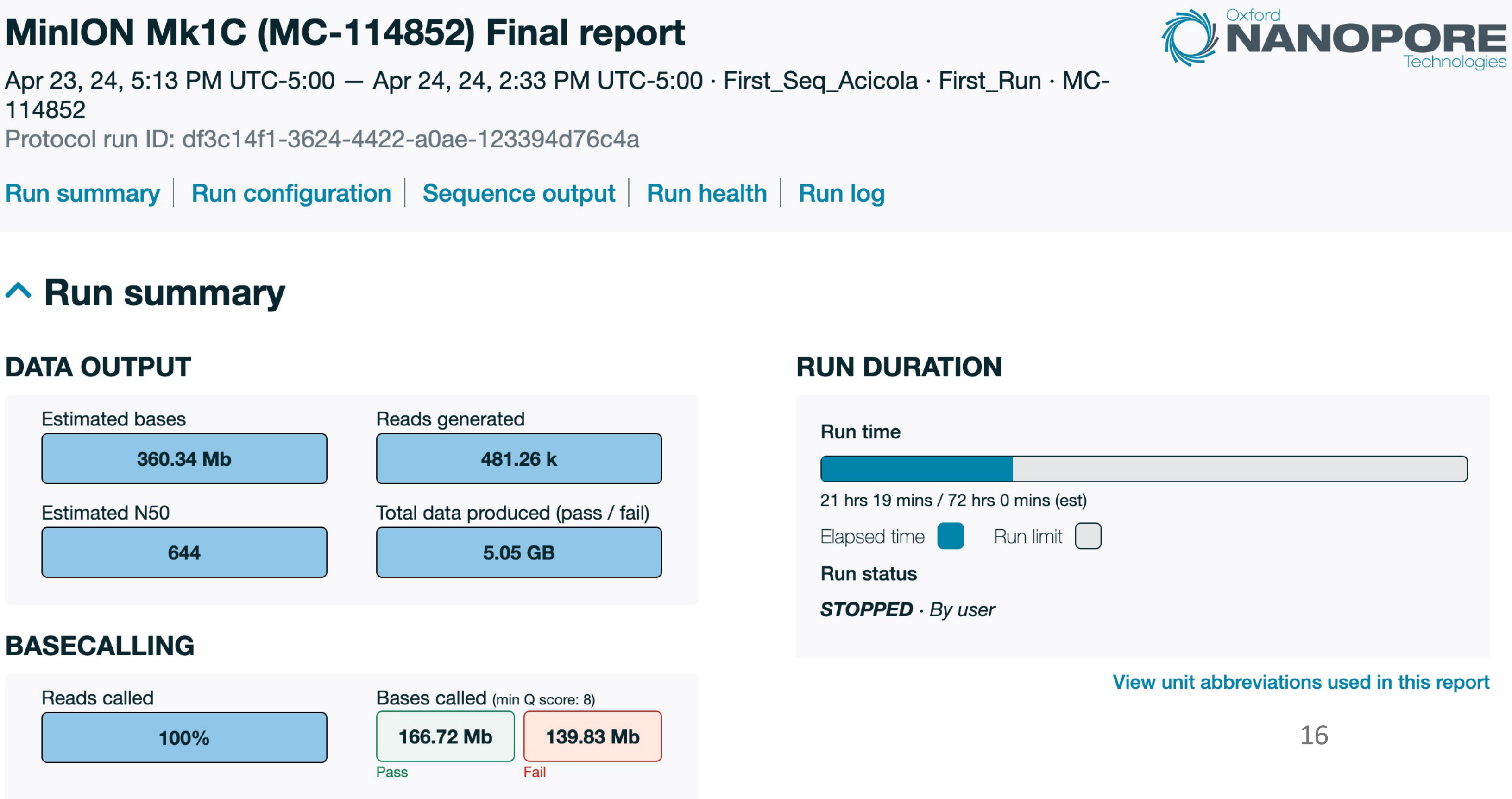
First_Seq_Acicola

First_Seq_Acicola Active

Reads 479.54 k   Estimated / basecalled bases 359.1 Mb / 303.8 Mb   Active runs 1   Total runs 1

Run controls

Position	Flow cell ID	Sample ID	Health	Available pores	Run time	Run state
MC-114852	FAW67448	First_Run		70 / 2048	21h 5m / 3d 0h	Active



MinION Mk1C (MC-114852) Final report

Apr 23, 24, 5:13 PM UTC-5:00 — Apr 24, 24, 2:33 PM UTC-5:00 · First_Seq_Acicola · First_Run · MC-114852

Protocol run ID: df3c14f1-3624-4422-a0ae-123394d76c4a

Run summary

DATA OUTPUT

Estimated bases: 360.34 Mb

Reads generated: 481.26 k

Estimated N50: 644

Total data produced (pass / fail): 5.05 GB

RUN DURATION

Run time

21 hrs 19 mins / 72 hrs 0 mins (est)

Elapsed time ■ Run limit ☐

Run status

STOPPED · *By user*

BASECALLING

Reads called: 100%

Bases called (min Q score: 8): 166.72 Mb Pass, 139.83 Mb Fail

View unit abbreviations used in this report

Reads Quality Output

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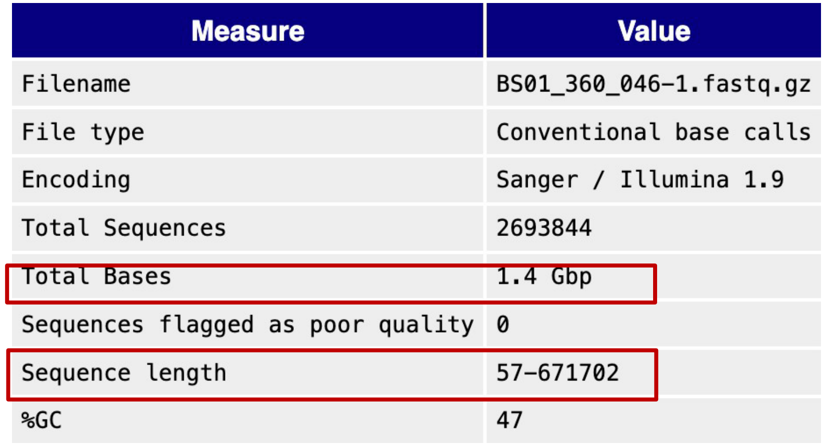
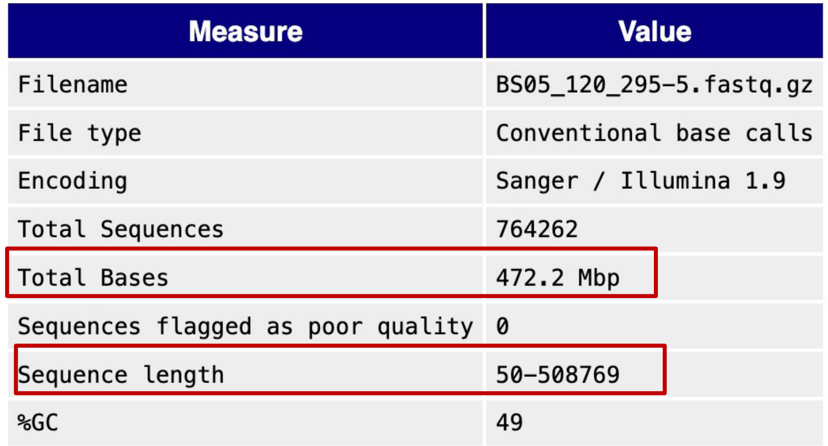
Initial outputs have high quality average but disparate total bases

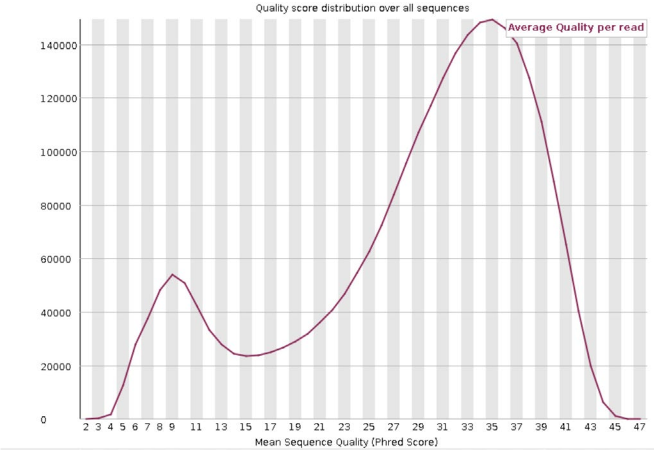
Summary output for two samples

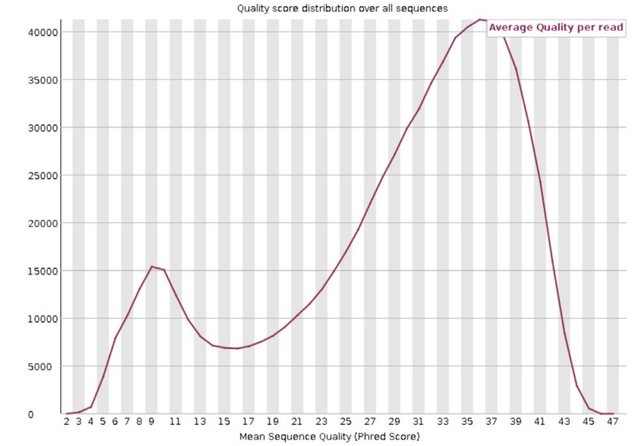


Measure	Value
Filename	BS01_360_046-1.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	2693844
Total Bases	1.4 Gbp
Sequences flagged as poor quality	0
Sequence length	57-671702
%GC	47



Measure	Value
Filename	BS05_120_295-5.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	764262
Total Bases	472.2 Mbp
Sequences flagged as poor quality	0
Sequence length	50-508769
%GC	49

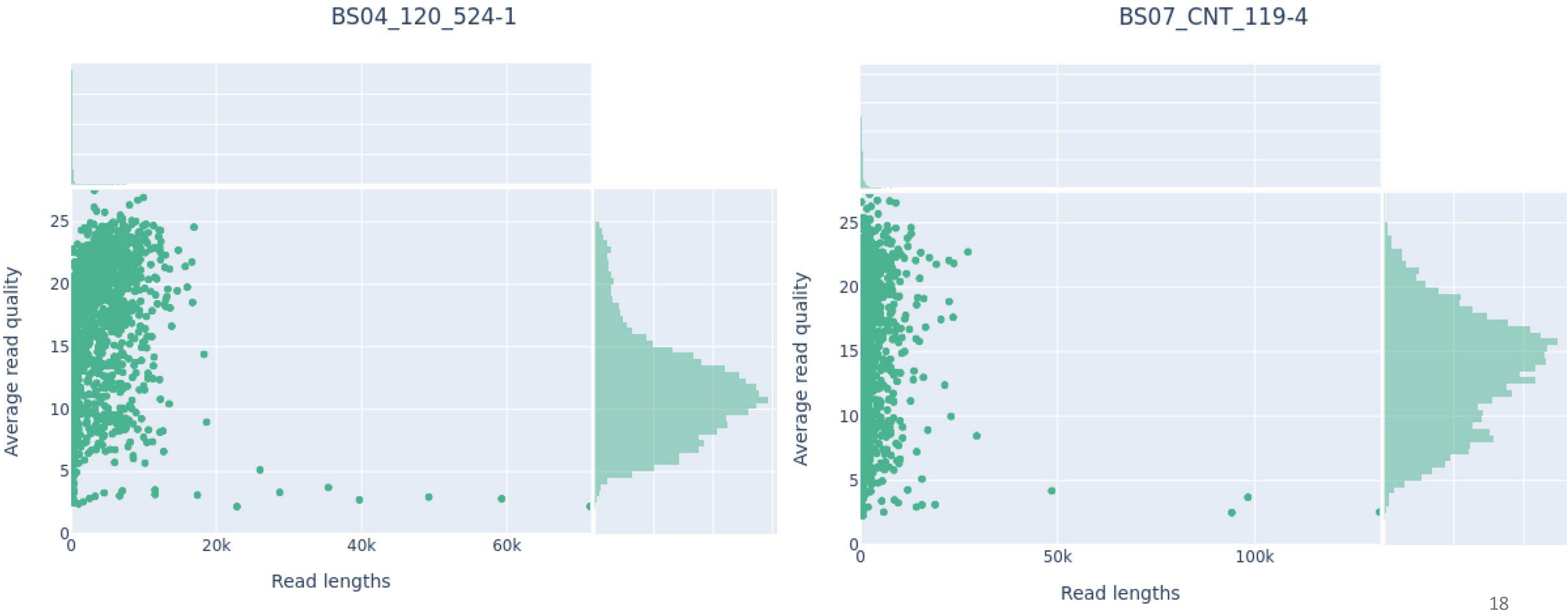




Frequency of quality scores per sequenced read

Reads Quality and Length Output

Initial outputs have expected distribution of read lengths



Future Directions

Next steps: sequencing, bioinformatics, and analyses

Complete sequencing

Assembly

Annotation

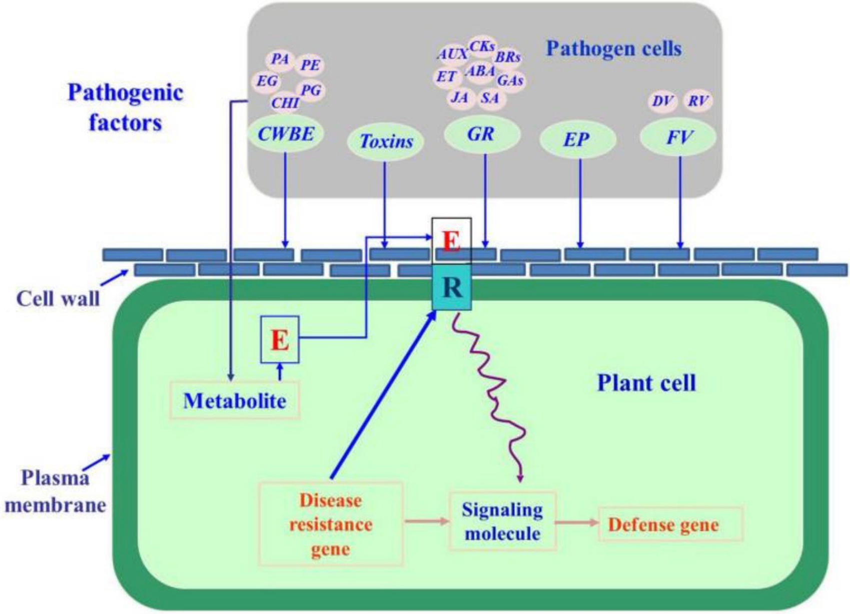
Call genome variants

Quantify the genetic variation and characterize population structure

Understand how *L. acicola* is spread and distributed regionally among stands, and make predictions about *L. acicola* effects based on this structure and identified variants of concern

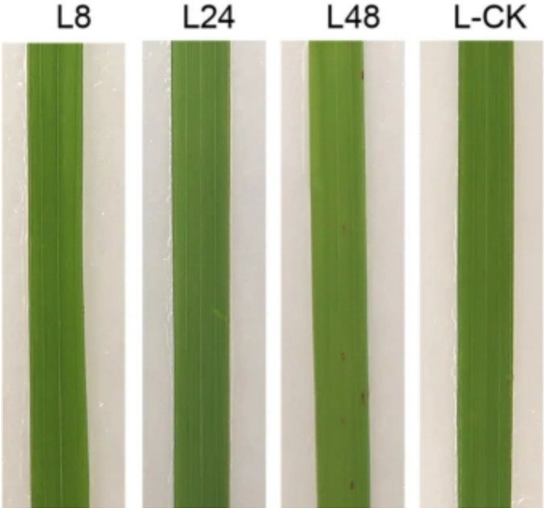
2. Identification and quantification of Pathogenic related genes in *L. acicola*

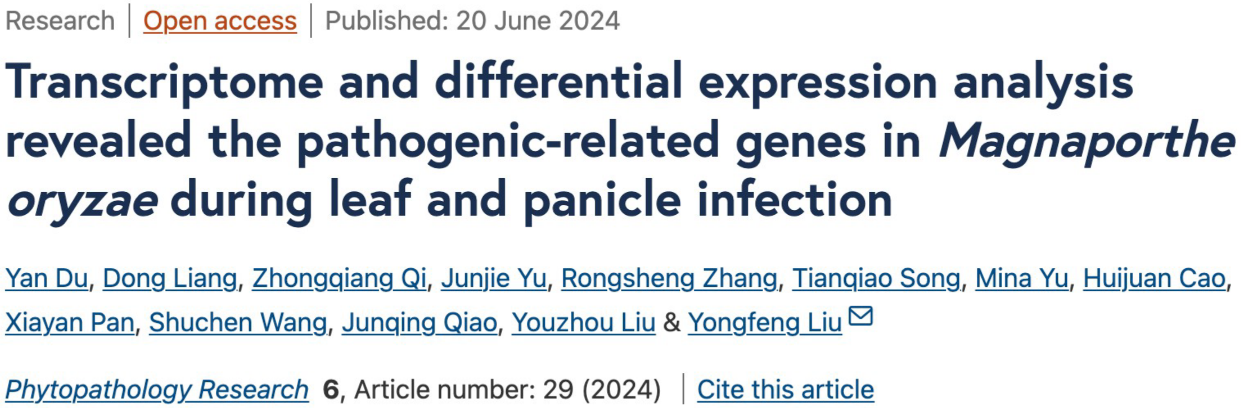
- The virulence and pathogenicity of plant pathogens are influenced by their ability to evade or suppress the plant's immune responses.
- Plant pathogenic fungi, secrete mycotoxins that act as virulence factors, aiding in the invasion of plant hosts by suppressing host resistance mechanisms
- These mycotoxins have distinct biosynthetic pathways, such as Dothistromin in Dothistroma and LA I & LA II in *L. acicola*
- Understanding the molecular mechanisms of *L. acicola* virulence is crucial for developing effective management strategies.



Transcriptome Analysis of Pathogenicity-Related Genes

Transcriptomes enable us to reveal infection-specific expression of *M. oryzae* genes in leaf and neck tissues



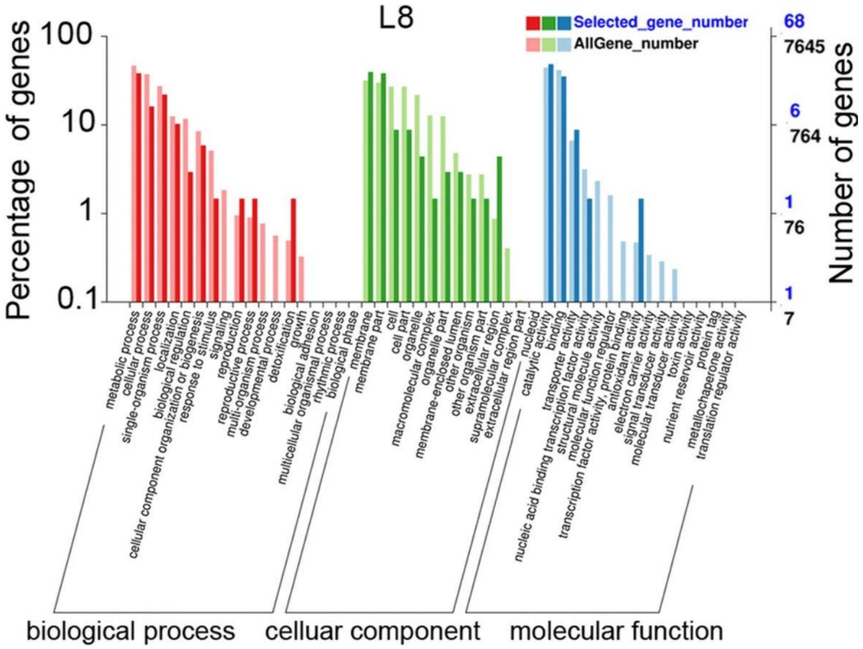


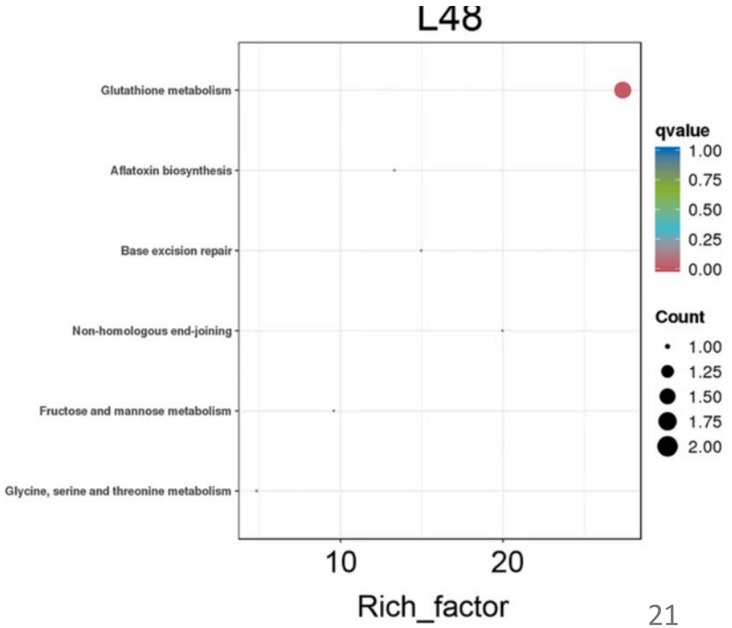
Research | Open access | Published: 20 June 2024

Transcriptome and differential expression analysis revealed the pathogenic-related genes in *Magnaporthe oryzae* during leaf and panicle infection

Yan Du, Dong Liang, Zhongqiang Qi, Junjie Yu, Rongsheng Zhang, Tianqiao Song, Mina Yu, Huijuan Cao, Xiayan Pan, Shuchen Wang, Junqing Qiao, Youzhou Liu & Yongfeng Liu ✉

Phytopathology Research **6**, Article number: 29 (2024) | Cite this article





Objectives

Q1. Identify and characterize differentially expressed genes in *L. acicola* isolates using RNAseq, with a focus on biosynthetic genes responsible for mycotoxin production

We hypothesize upregulation of toxins related genes will positively correlate with disease severity

Methods

RNA Extraction of *L. acicola* isolates

RNA sequencing (NGS)

Quality Control

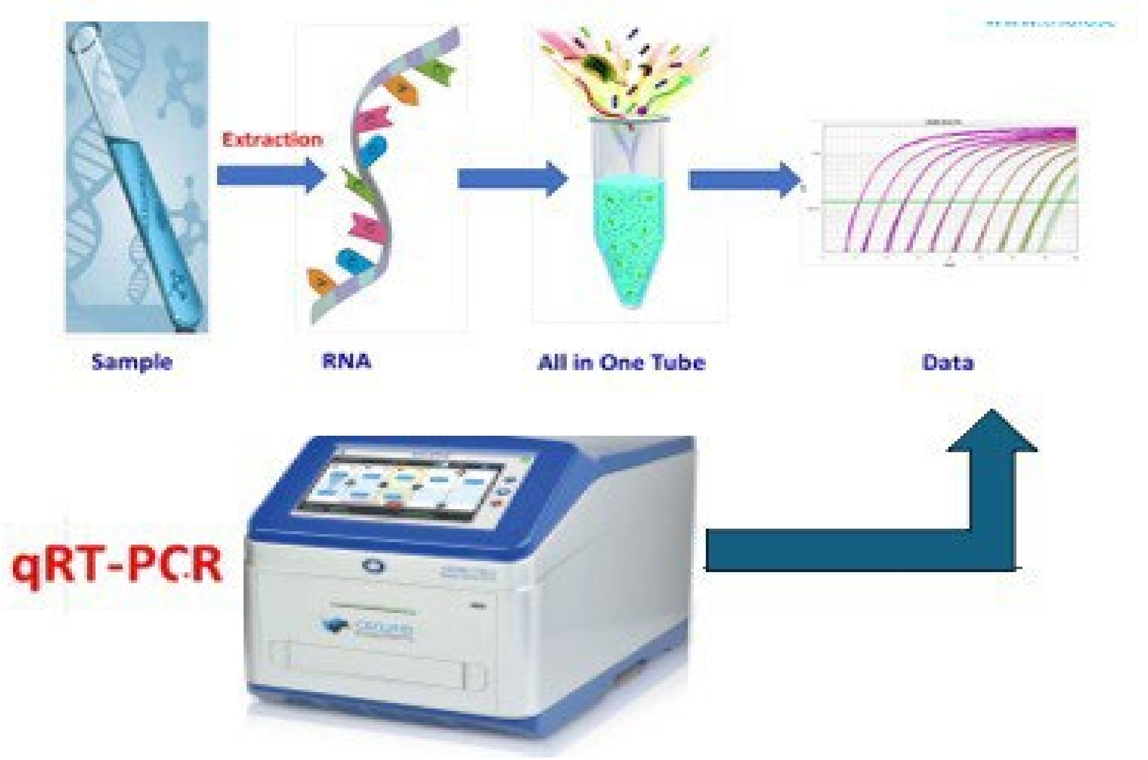
Transcriptome profilling

Differential Gene Expression

Future directions

- We expect to find correlation between upregulation of toxins related genes to disease severity among regions

- We expect to find similar patterns among similar geographic regions



Azure Cielo qPCR machine

3. Fungal load and pathogenicity of *L. acicola*

- Quantifying fungal biomass
- Disease severity information on host plant
- Phenotypic characteristics of host Plant

Accurate detection, diagnosis, and management of fungal disease outbreaks depends on understanding the quantity of fungus that is needed for detection and how this predicts disease progression

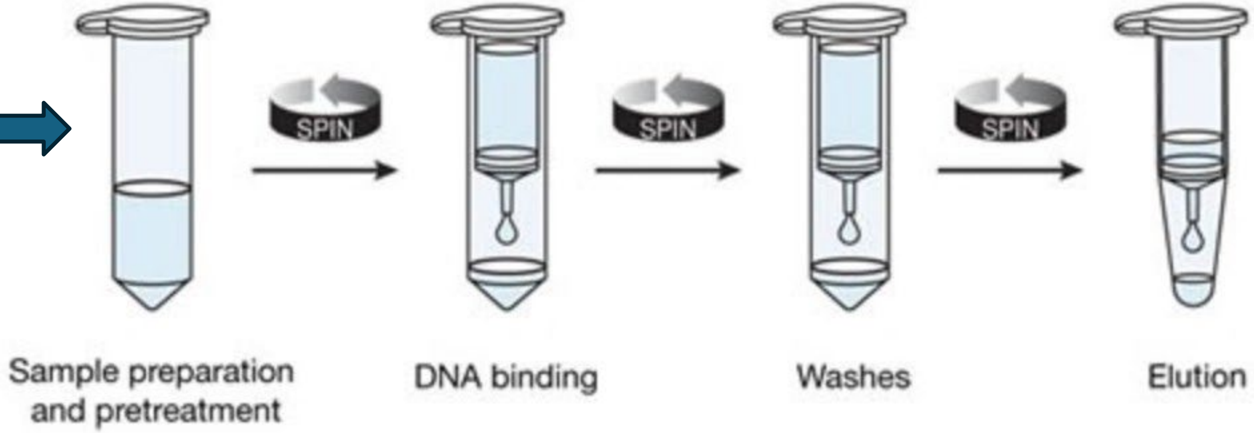
Objectives

Q1. We will quantify *L. acicola* load and compare values to disease severity changes over time

Q2. We will compare fungal loads between long leaf and loblolly, to understand species specific differences in infection progression, association between habitats

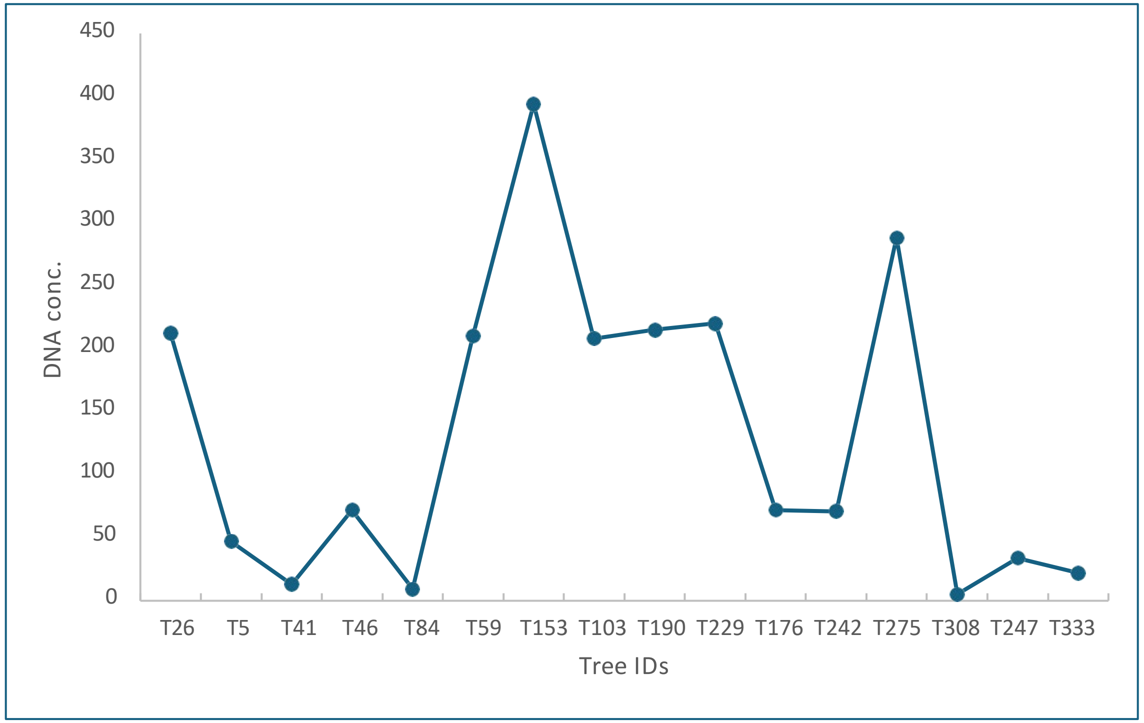
Methods

- Total DNA extraction from symptomatic needle tissues using Qiagen DNeasy pro kit

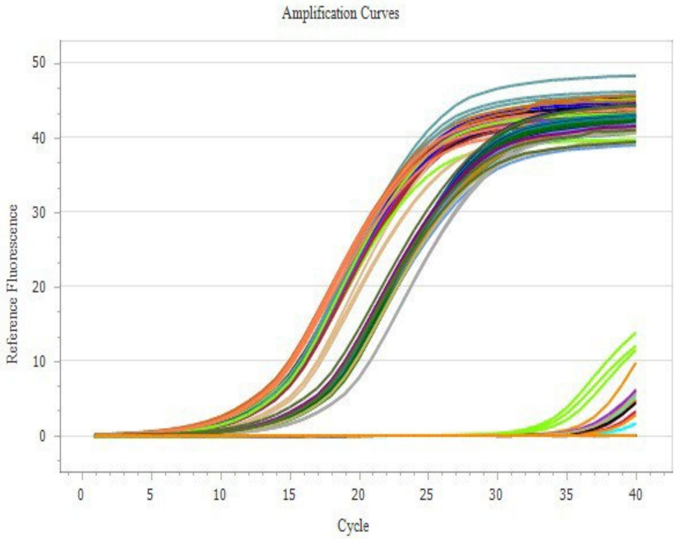


Target region is LAtef (TEF region)
and ITS for normalization

Preliminary results

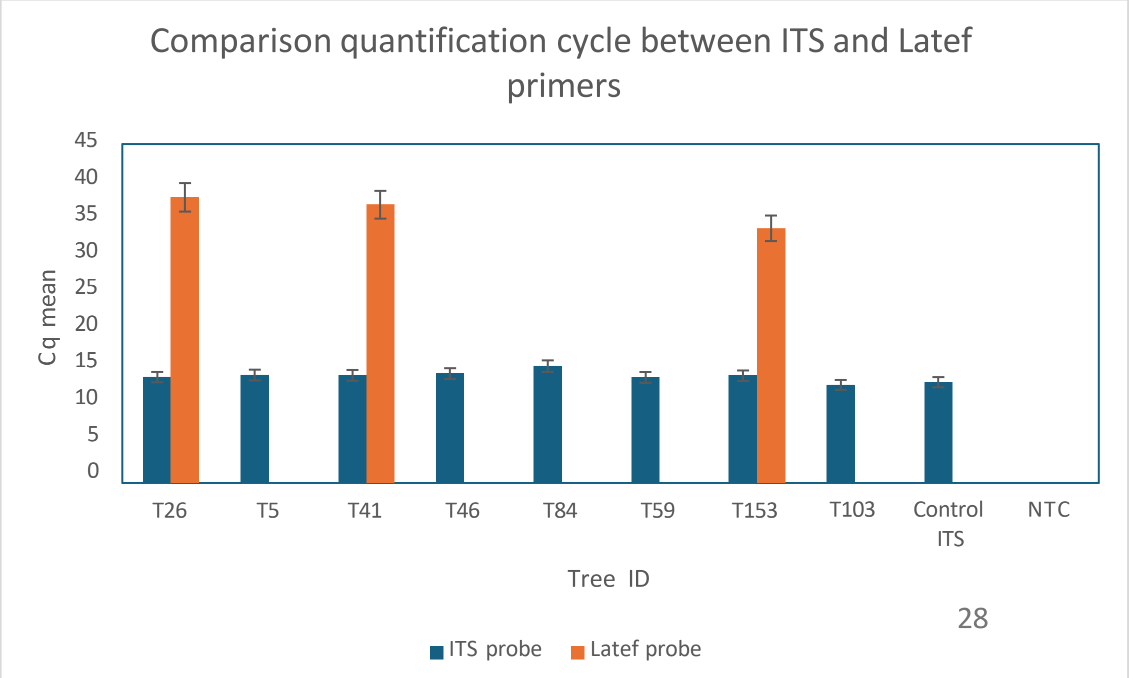


Variation in DNA Concentration across different Tree IDs measured in nanograms per microliter



Samples

Fluorescence increasing as the DNA target is detected



Higher Cq value indicate lower DNA of the fungal in plant, Samples are measured in three technical replicates.

Thank you to funders and collaborators!

AUBURN UNIVERSITY

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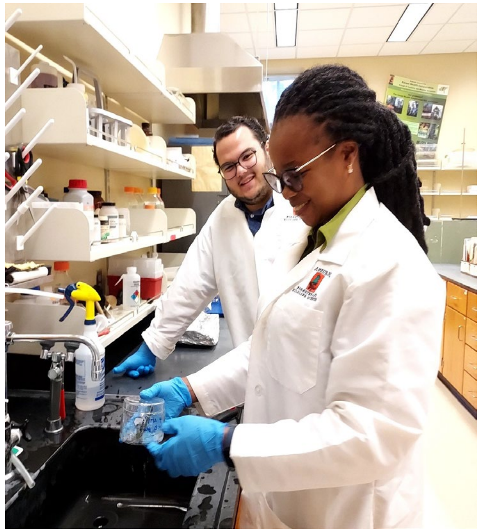
College of Forestry
and Wildlife Sciences

REGIONS
Kris Bradley



Questions?

Stallworth Land Company
Long Leaf Land & Timber Company
Osko Forest - Dr. Glenn Glover

Forest Health Dynamics Lab
Dr. Lori Eckhardt

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