Identification and Distribution of Fungal Pathogens associated with Loblolly Pine Defoliation and Mortality in the Southeastern U.S.

Presented By

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Agenda

Chapter I: Introduction and review of literature

Chapter II: Isolation and identification of fungi associated with loblolly pine needle defoliation and mortality in the southeastern U.S.

Chapter III: Needle pathogen, *Lecanosticta acicola*, effects on *Pinus taeda* needle and shoot lengths

Chapter IV: *Lecanosticta acicola* impacts on foliar nutrient contents and total phenolics in *P. taeda* needles

Chapter V: Prediction of loblolly pine defoliation severity associated with changes in pathogen pressure in response to climate change in the southeastern U.S.

Chapter VI: Future directions of the study

Motivation of the study

Loblolly pine in the Southeastern U.S.

- Important timber species
- Periodic defoliation and mortality
- Thousands of hectares infected
- Increasing annual reports

Disease symptoms

- Yellowing, browning and defoliation
- Completely empty tree crown
- Current year needles infected

Support of the study

- No evidence of exposure to CFC's and SO_2
- Nutrient deficiencies

Introduction

Pine forests and industrial wood plantations in the Southeastern U.S.

- More than $11 billion

- Sustainability and profitability

- Non-native insect pests and pathogens

- Movement of native forest pests

- Damage approximately $4.2 billion annually

Introduction

Needle diseases

- Temperature and moisture
- Reproduction, fungal spread and infection
- Fungal richness and abundance

Climatic stressors

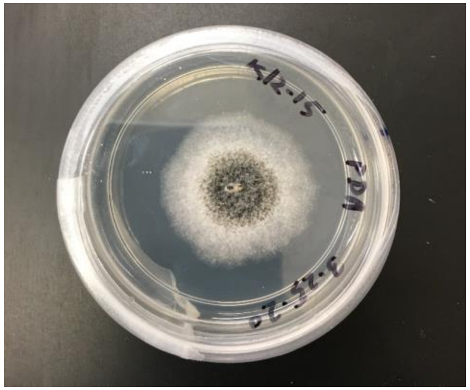
- Increasing temperature and precipitation
- Changing interactions
- Changes in disease impacts

For example,

- *Phytophthora pluvialis* in New Zealand
- *Phytophthora pinifolia* in Chile

Chapter II

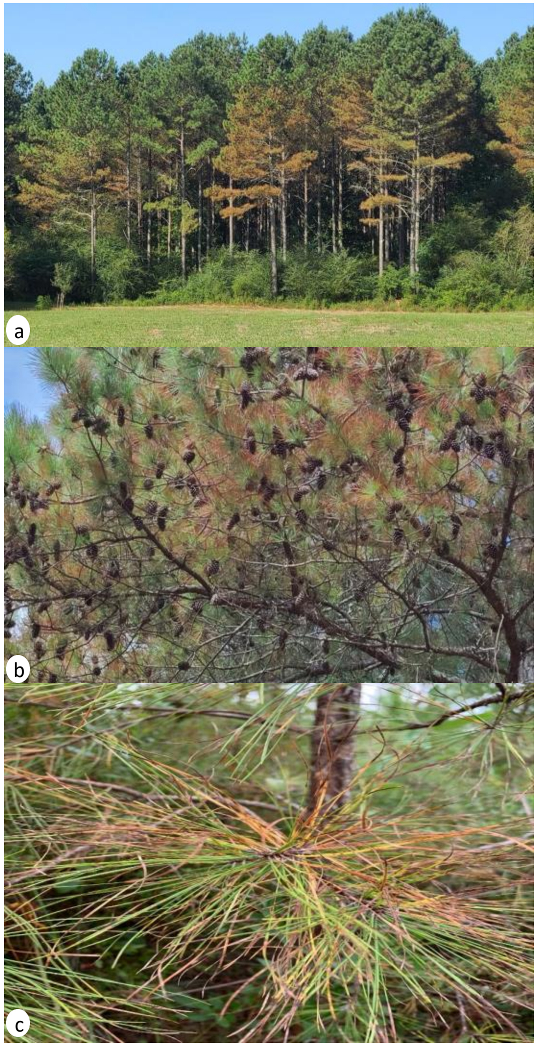
Isolation and Identification of Fungi associated with Loblolly Pine Defoliation and Tree Mortality in the Southeastern U.S.



Introduction

Loblolly pine defoliation and tree mortality

- First contacted to FHDL in 2013
- Successive defoliation
- Needle mortality and mature tree death
- Chlorosis, needle necrosis and premature defoliation
- Spread to adjacent areas
- Widespread mortality at summer 2018
- Disease patterns was ambiguous
- Not all adjacent loblolly stands are infected
- More than 25,000 hectares were reported

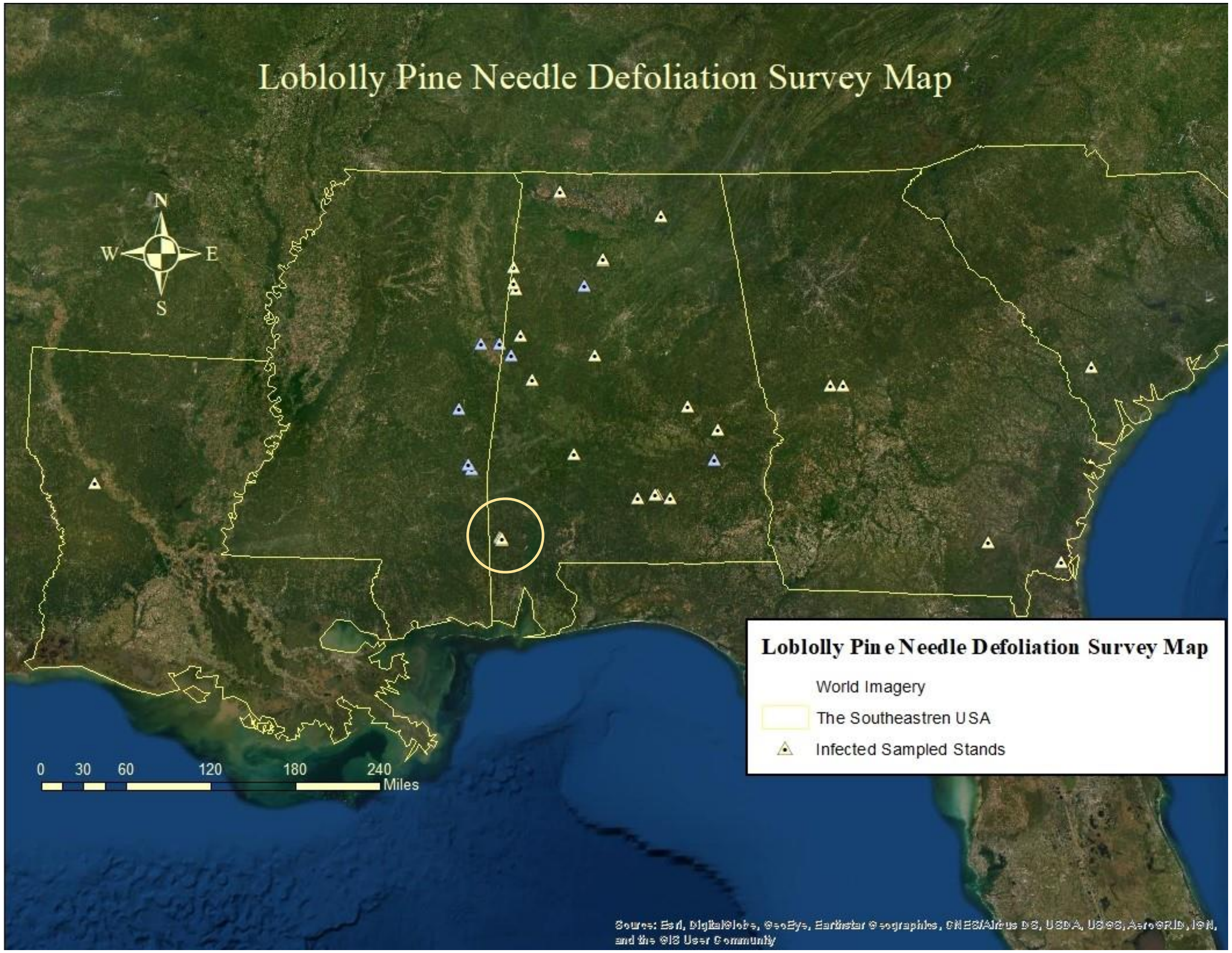


Objectives

To isolate and identify the causal agent(s) associated with loblolly pine defoliation and mortality in the Southeastern U.S.

To identify morphological and genetic diversity of fungi associated with loblolly pine defoliation and mortality in the Southeastern U.S.

Study Area



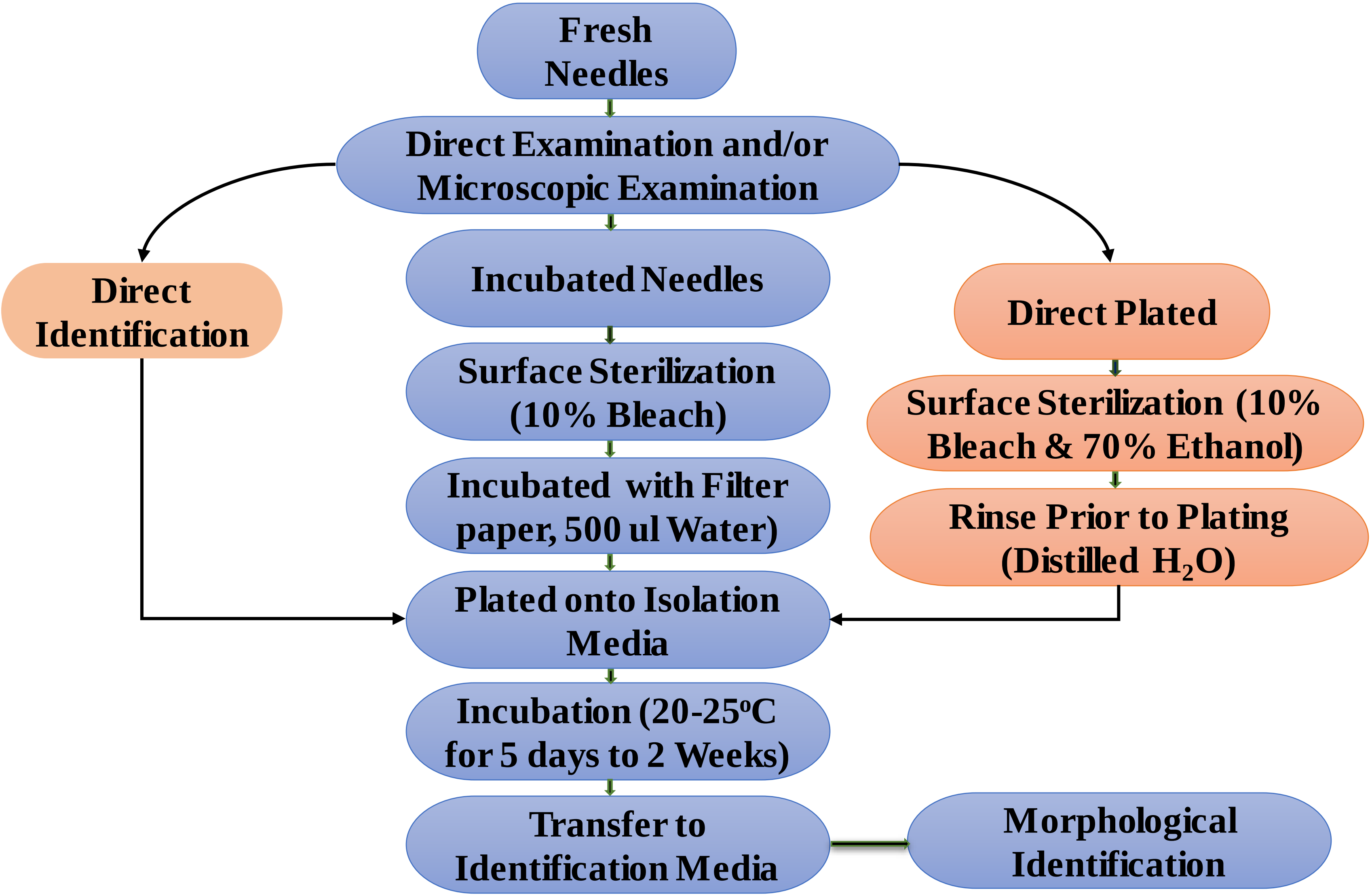
Map showing permanent and surveyed stands in South Carolina, Georgia, Alabama, Mississippi, and Louisiana from 2019 to 2021 from surveys and the permanent study area

Study Area

Sporulation period of five needle pathogens

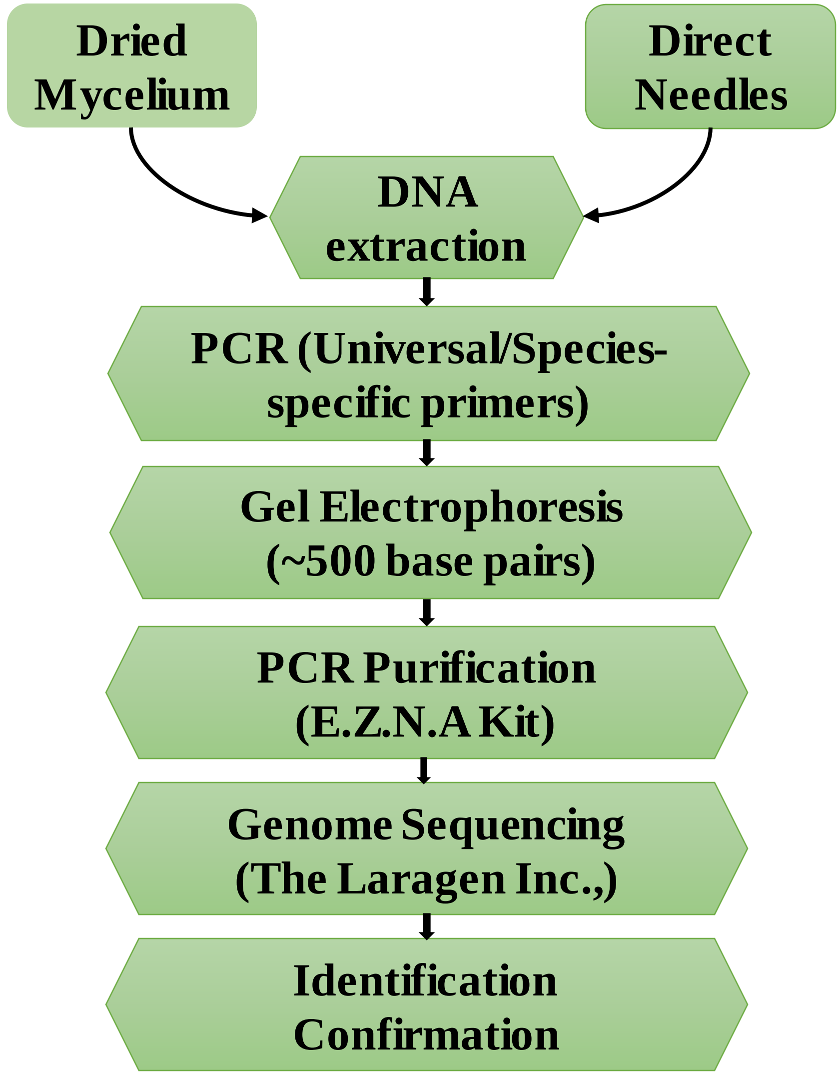
Needle Disease	Causal Agent	Host	Sporulation Period
Phytophthora needle blight	*Phytophthora* spp.	Pines, Oak, Douglas fir,	May to November
Brown spot needle blight	*Lecanosticta acicola*	Over 53 different pine species	March to October
Dothistroma needle blight	*D. septosporum* *D. Pini*	82 Pinus taxa	Late summer to fall (August-October)
Lophodermium needle cast	*L. seditiosum* *L.* spp.	Scots, Austrian and Red pine	Late summer (August-September)
Coleosporium needle rust	*Coleosporium* spp.	2 or 3-needled Pines	Spring (March-May)

Materials & Methods



Cultural methods of fungi identification

Materials & Methods



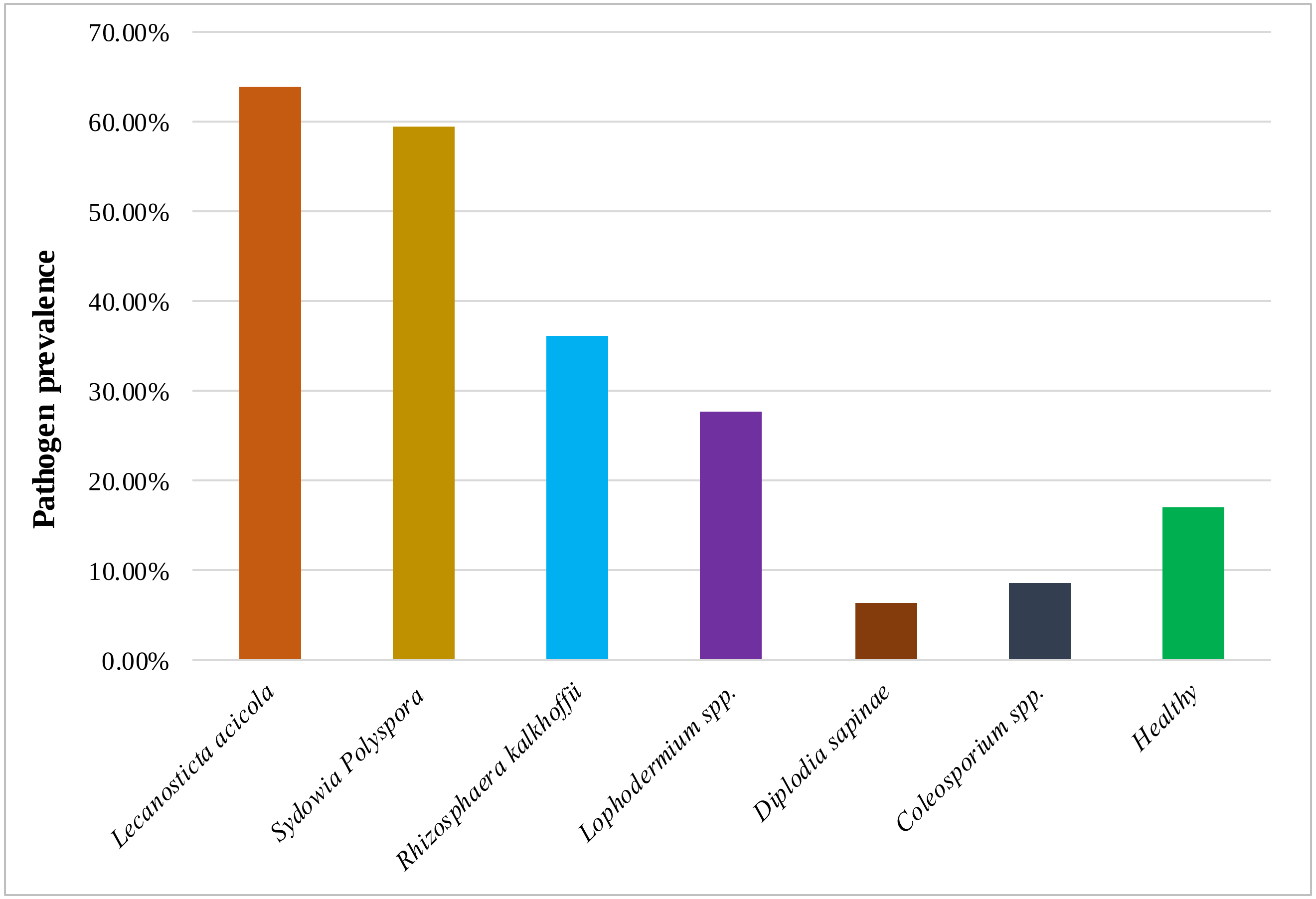
Molecular methods of fungi identification

Materials & Methods

Phylogenetic analysis

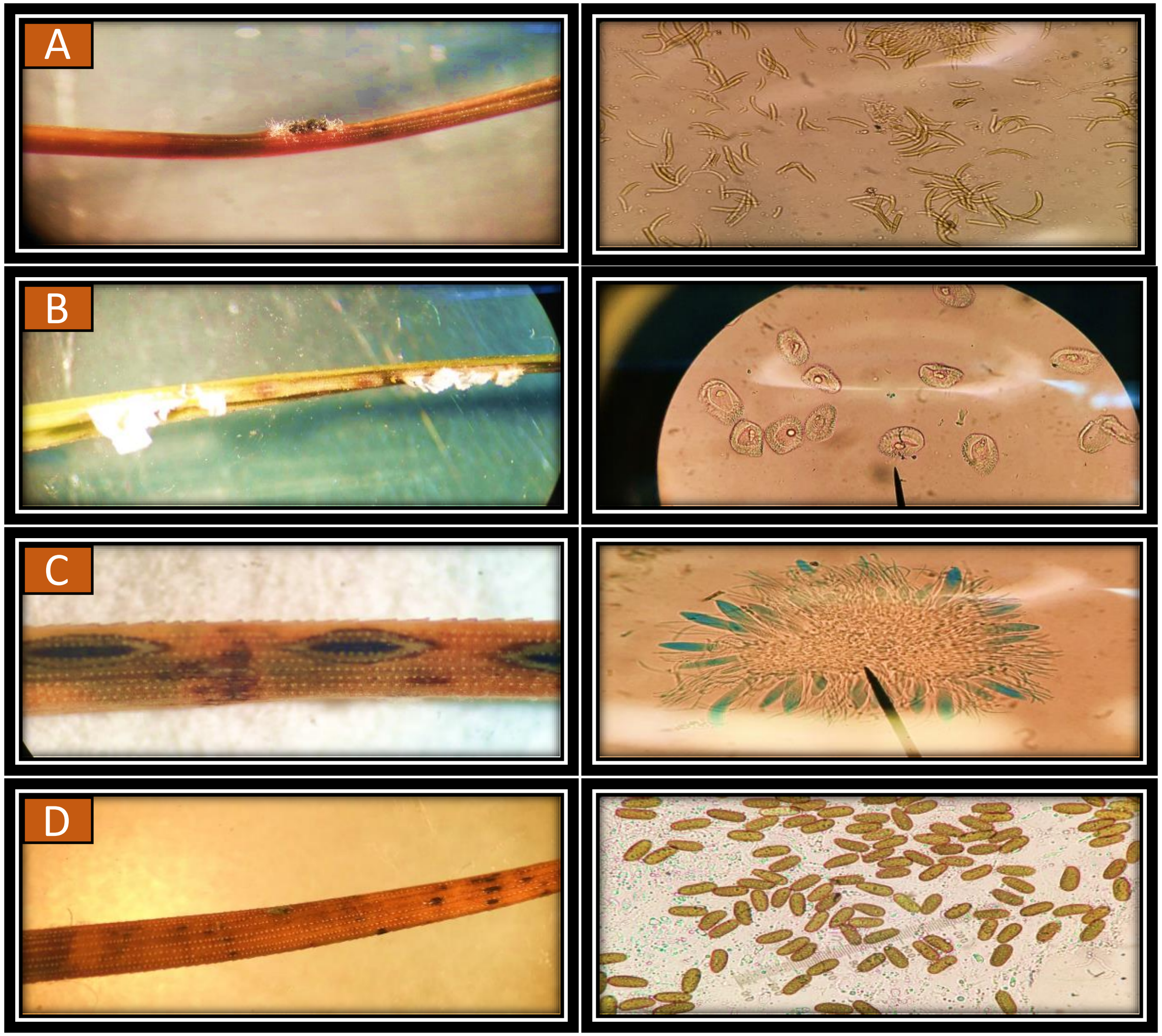
- Laragen Inc., Biotechnology for sequencing
- Raw sequences were edited and adjusted
- Bioedit software and deposited in GenBank and BankIT
- MEGA version 4.0 software
- Alignment were completed
- Neighbor Joining (NJ) and Maximum Likelihood (ML) methods
- 1000 bootstrap replications were performed

Results



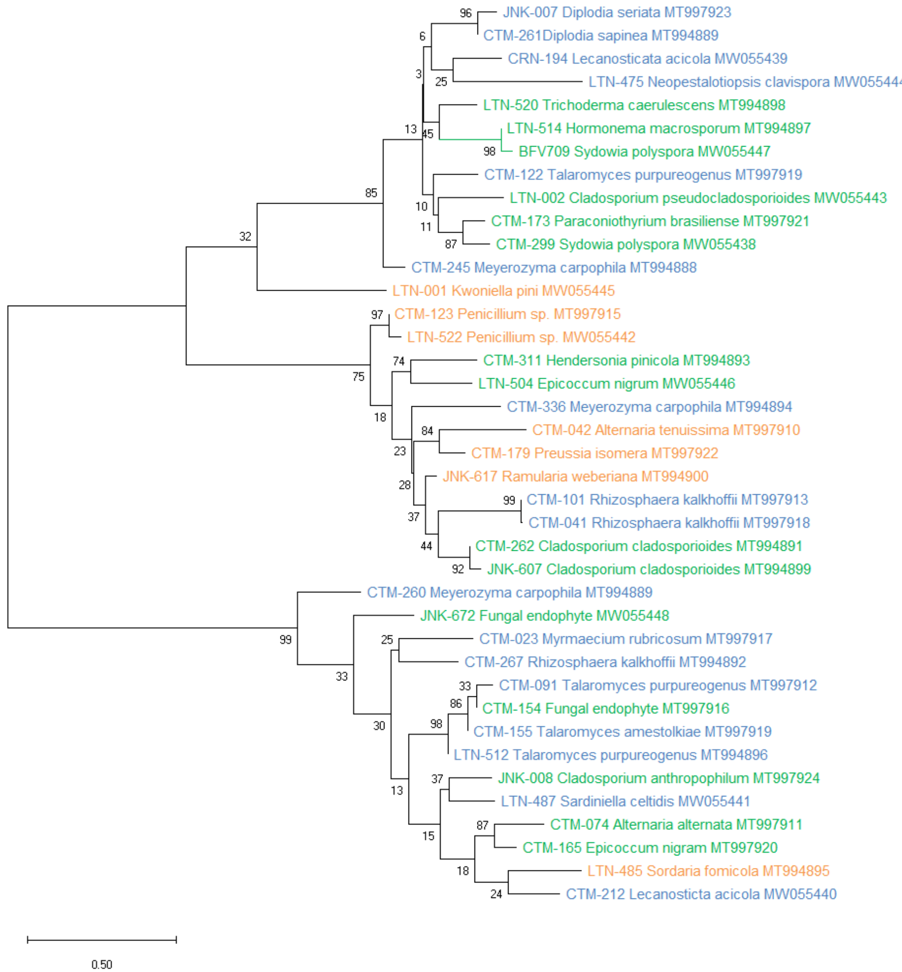
Pathogen prevalence of *Lecanosticta acicola*, *Sydowia polyspora*, *Rhizosphaera kalkhoffii*, *Lophodermium* spp., *D. sapinae*, *Coleosporium* spp. and healthy sites

Results



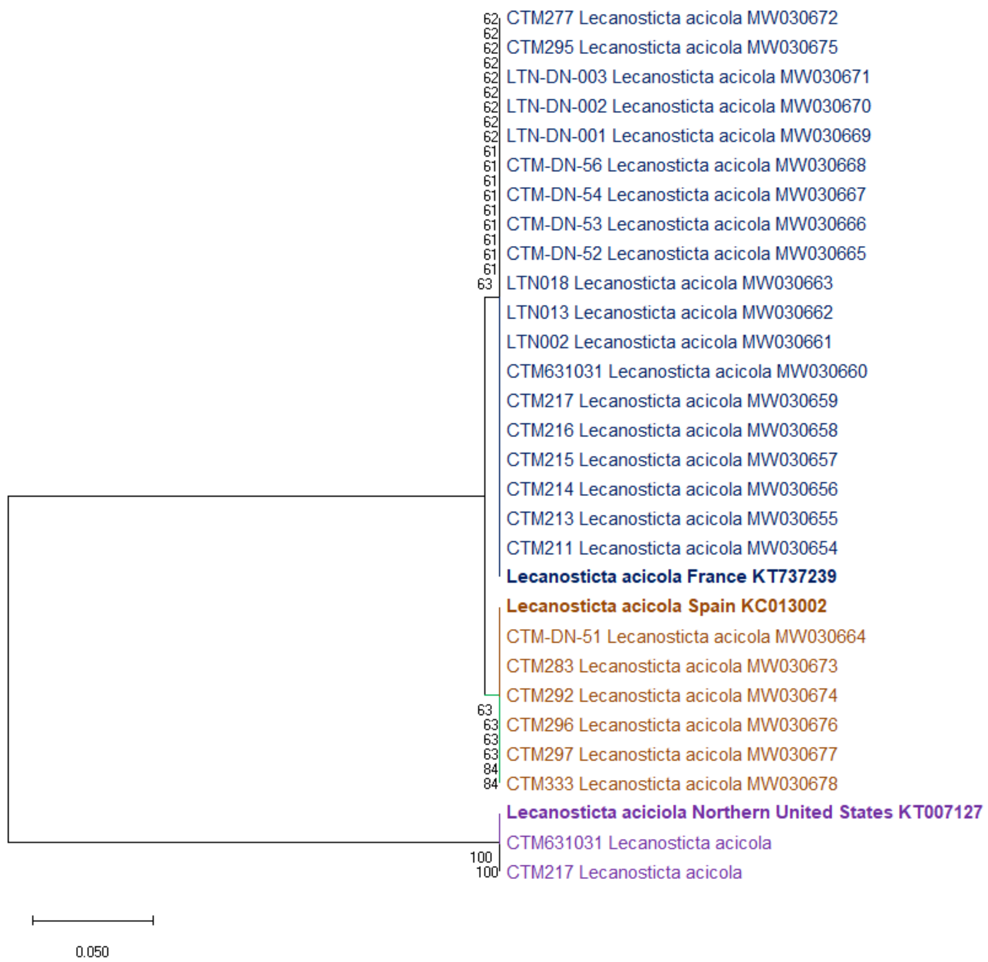
Disease symptoms and reproductive structures of (A) *L. acicola* (B) *Coleosporium sp.* (C) *Lophodermium sp.* & (D) *D. sapinea* on loblolly pine

Results



Neighbor-joining phylogenetic tree based on ITS1 and ITS4 sequences of fungi recovered from loblolly pine needles in the study

Results



Maximum likelihood phylogenetic tree representing *L. acicola* and its associated lineages

Results

Brown spot needle blight (BSNB) fungus, *Lecanosticta acicola*

- Predominant pathogen in the southeastern U.S.
- Three distinct lineages
- Asexual state recovered (Alabama and Mississippi)
- Black to olive green mucilaginous conidiomata
- Fusiform to cylindrical with straight to curved conidia
- Septation 2-4 with truncate base and rounded apex
- Positive for a single mating type loci, MAT-1-1

Conclusions

First report of *Lecanosticta acicola* associated with loblolly pine defoliation and tree mortality

- Central, Southern, Northern, and Southwestern counties

- Less genetically diverse

Sydowia polyspora

- Endophytic to pathogenic

- Disease severity in the infected stands

Genetic and environmental factors

- Further investigation

Chapter III

Needle Pathogen, *L. acicola*, Effects on *P. taeda* Needle and Shoot Lengths

Introduction

Healthy trees

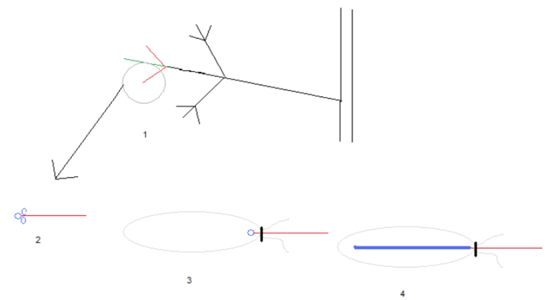
- Robust shoot growth, long and lush green needles
- Optimal growth and productions

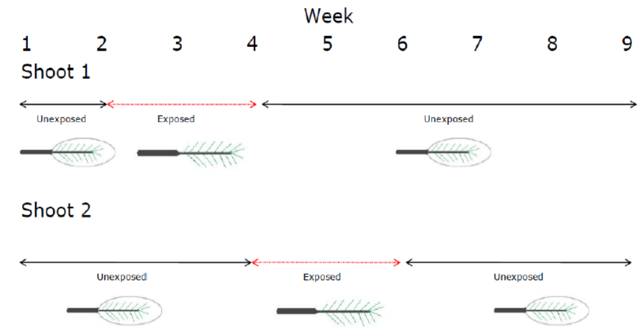
Shoot development in pine trees

- Long shoot and short shoot development
- Impacts of stress and growth promotion factors

Brown spot needle blight fungus, *L. acicola* impacts

- Needle and shoot sizes have not been assessed
- Disease progression



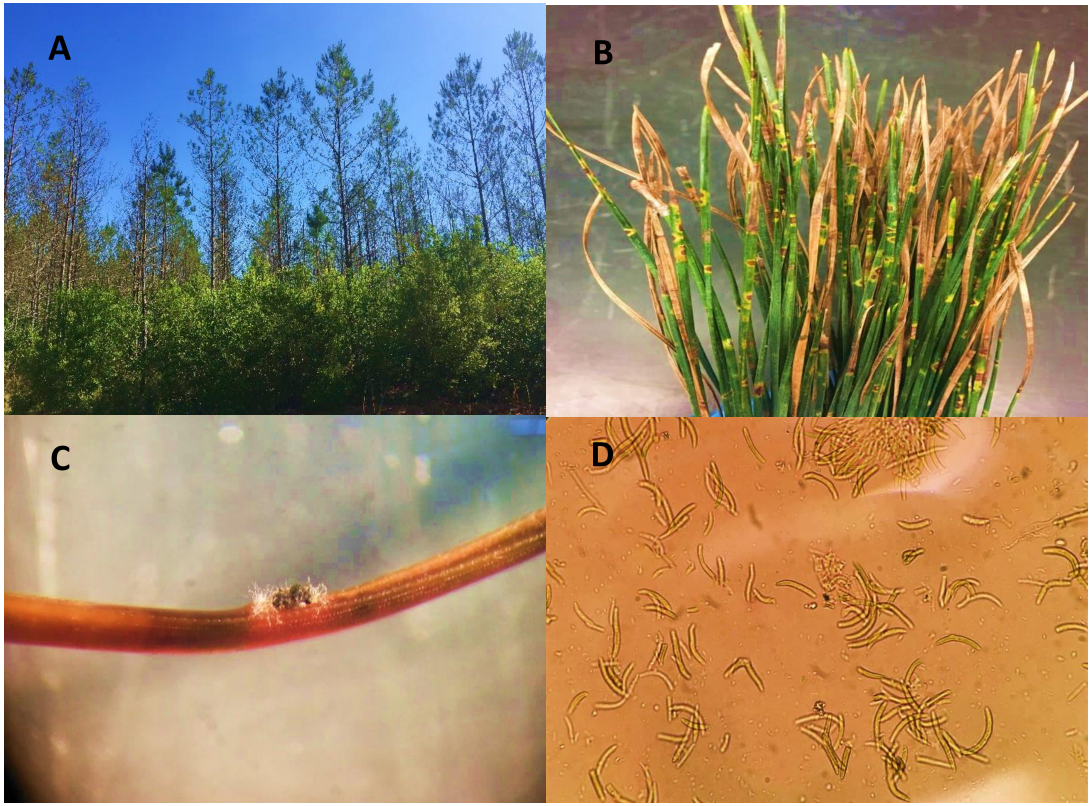


Objectives

To assess brown spot needle blight fungus, *Lecanosticta acicola* effects on shoot and needle lengths

To annually monitor loblolly pine health for chlorosis and defoliation in permanent study plots

Materials & Methods



Disease symptoms and reproductive structures showing (A) stands infected by brown-spot needle blight, note the thinning canopies (B) irregular frequent brown-spots surrounded by a yellow halo (C) black shiny fruiting body protruding needles & (D) microscopic banana-shaped septate conidia

Materials & Methods

Study design and data collection:

- Two infection levels

- Two whorl heights

- 28 low incidence and 33 high incidence trees

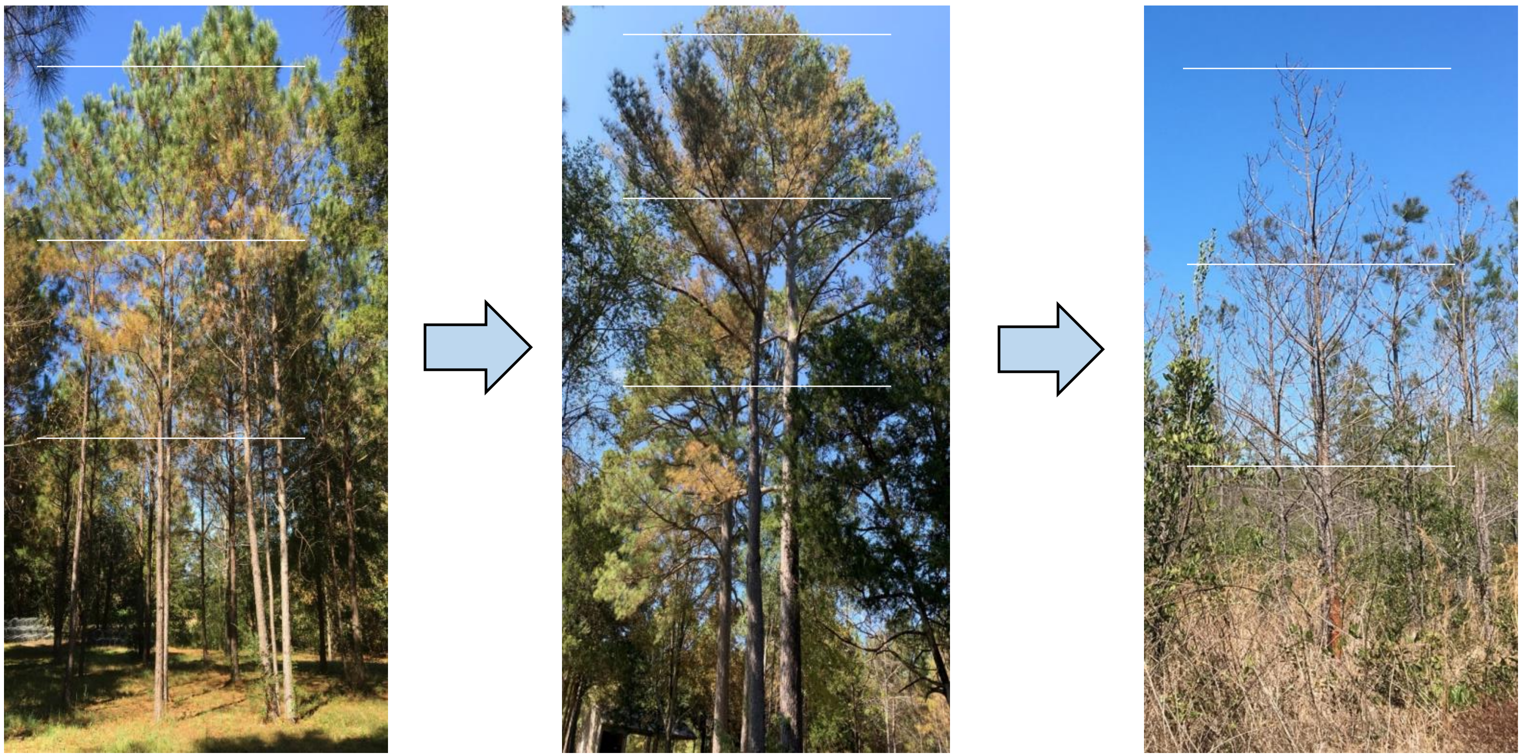
- 10 fascicles

- End of the growing season

- 2019 and 2020

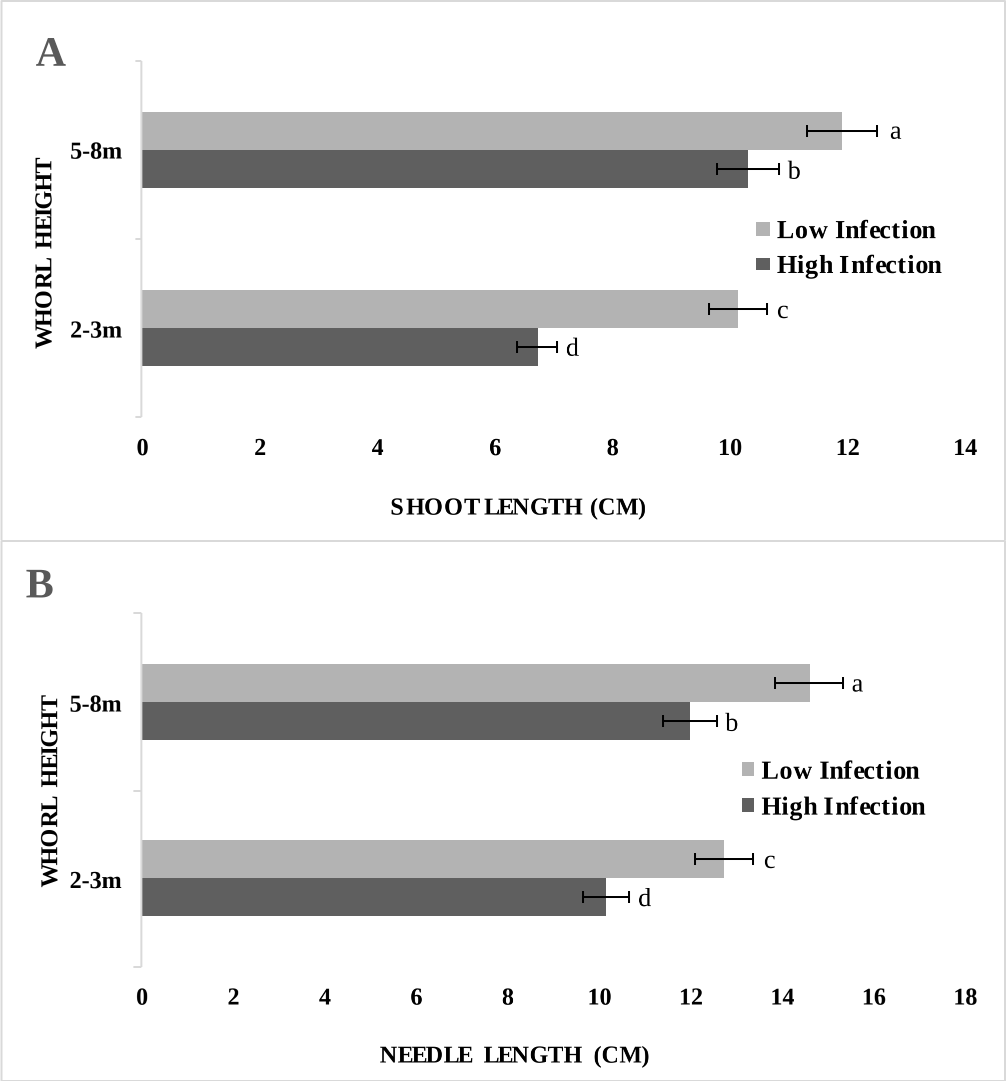
Loblolly Pine Health Monitoring

Tree health rating was done by visual inspection to determine disease severity as the proportion of the crown affected;



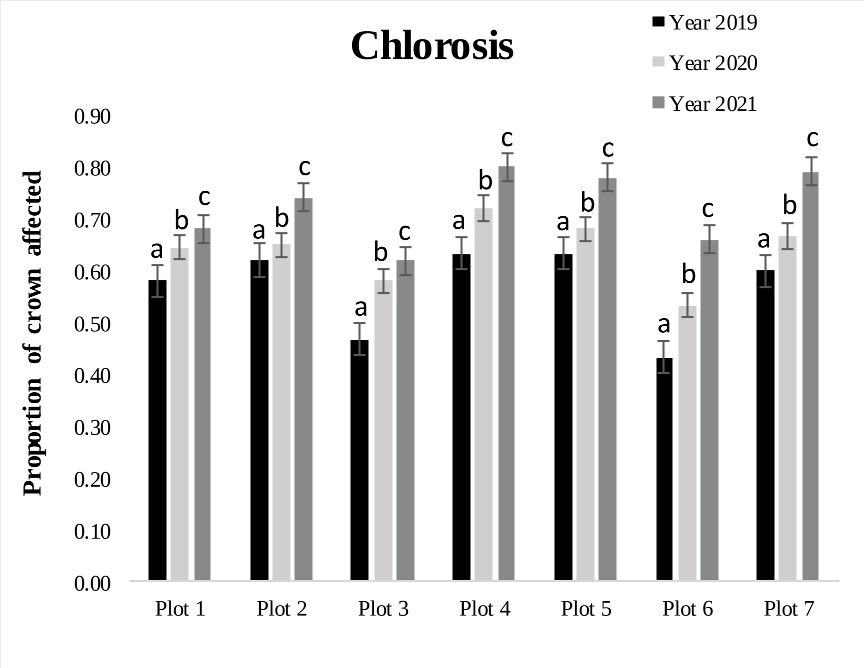
Data were analyzed using MS Excel 2010. Response variable was either "chlorosis rating per tree" or "defoliation per tree"

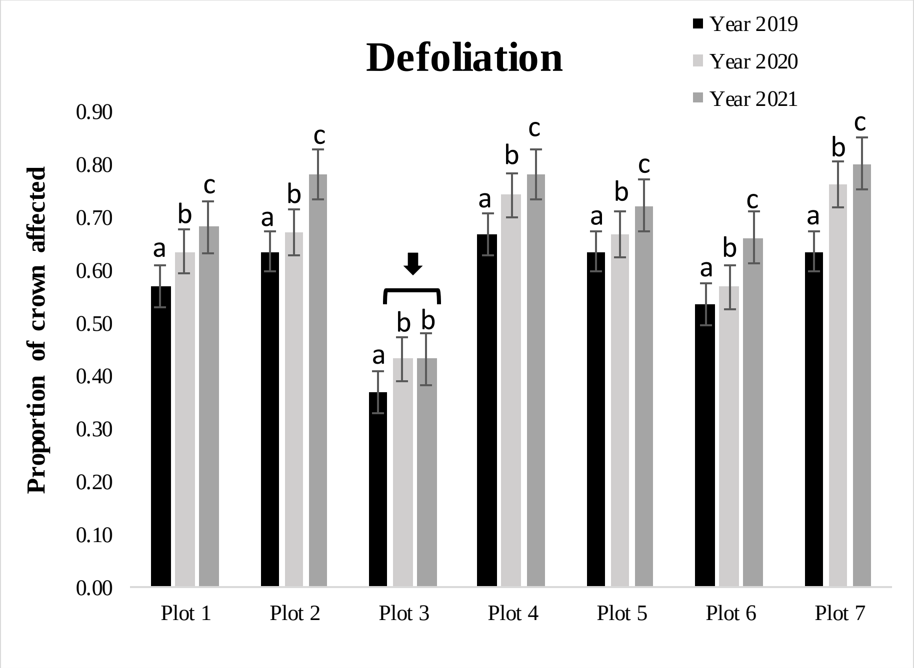
Results



Observed means and standard errors of (A) shoot length and (B) needle length

Results





Bar represents the mean chlorosis and defoliation of seventy tagged trees at seven long-term monitoring plots in Chatom, Washington County, Alabama in the summer of 2019, 2020 and 2021

Conclusions

Lecanosticta acicola infection

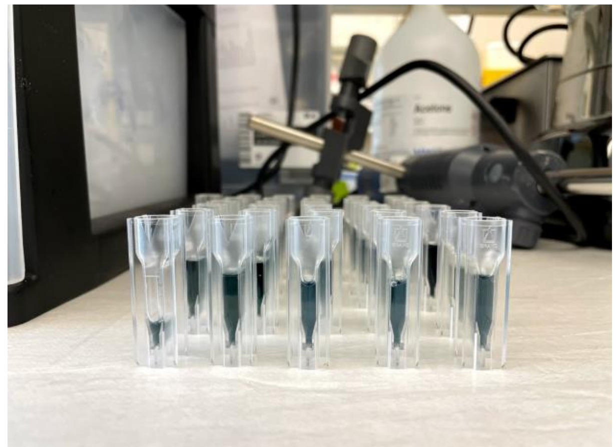
- Shoot and needle lengths reduction
- Whorl height and shoot and needle lengths
- Leaf mechanical support and physiological functions

Long-term monitoring of loblolly pine health

- Disease progression
- Air currents or rain-splash spores
- Healthy trees infected
- Unhealthy trees more chlorotic and defoliated

Chapter IV

Lecanosticta acicola Impacts on Foliar Nutrient Contents and Total Phenolics in *P. taeda* Needles



Introduction

Nutrient availability

- Tree growth and productivity
- Host-pathogen interactions
- Manipulate management decision

Brown spot needle blight (BSNB)

- Geographical settings, climate and other plantation attributes
- Little known about BSNB and interaction of nutrients

Conifers including loblolly pine

- Constitutive and inducible defenses to prevent attack from pathogens
- Defensive chemicals and resistance

Objectives

To determine the relationship between foliar nutrients and infection level to see how nutrients interact with *Lecanosticta acicola* severity

To evaluate the interactions between *L. acicola* severity and defensive chemical total phenolics in loblolly pine needles

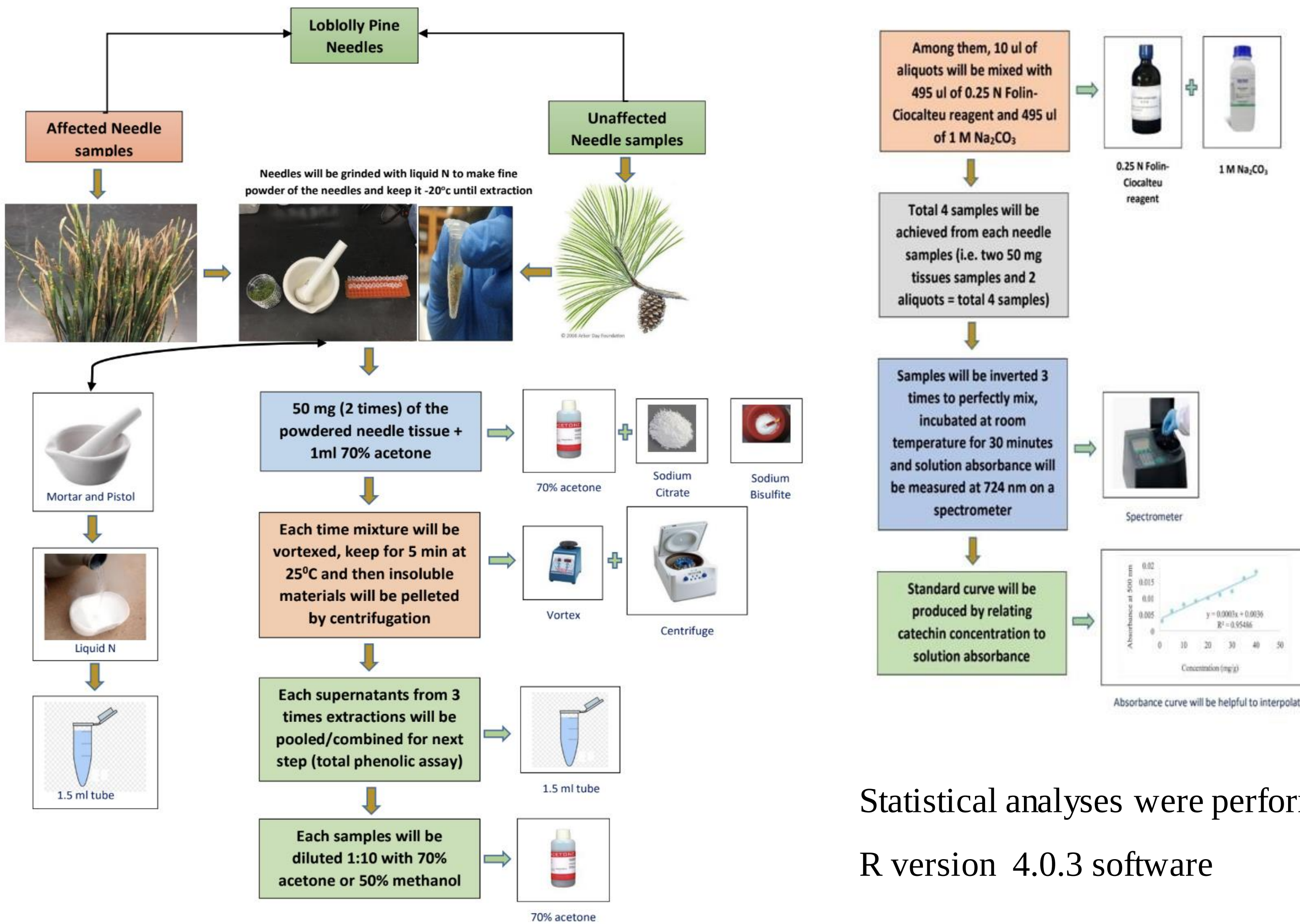
Materials & Methods

Study area, plant material collection:

- A 0.22 mag caliber rifle
- Destructive sampling of foliage
- Fifty trees sampled
- Fifty fascicles per tree
- Oven-dried at 70°C
- 0.5 mm mesh screen
- Waypoint Analytical Laboratory

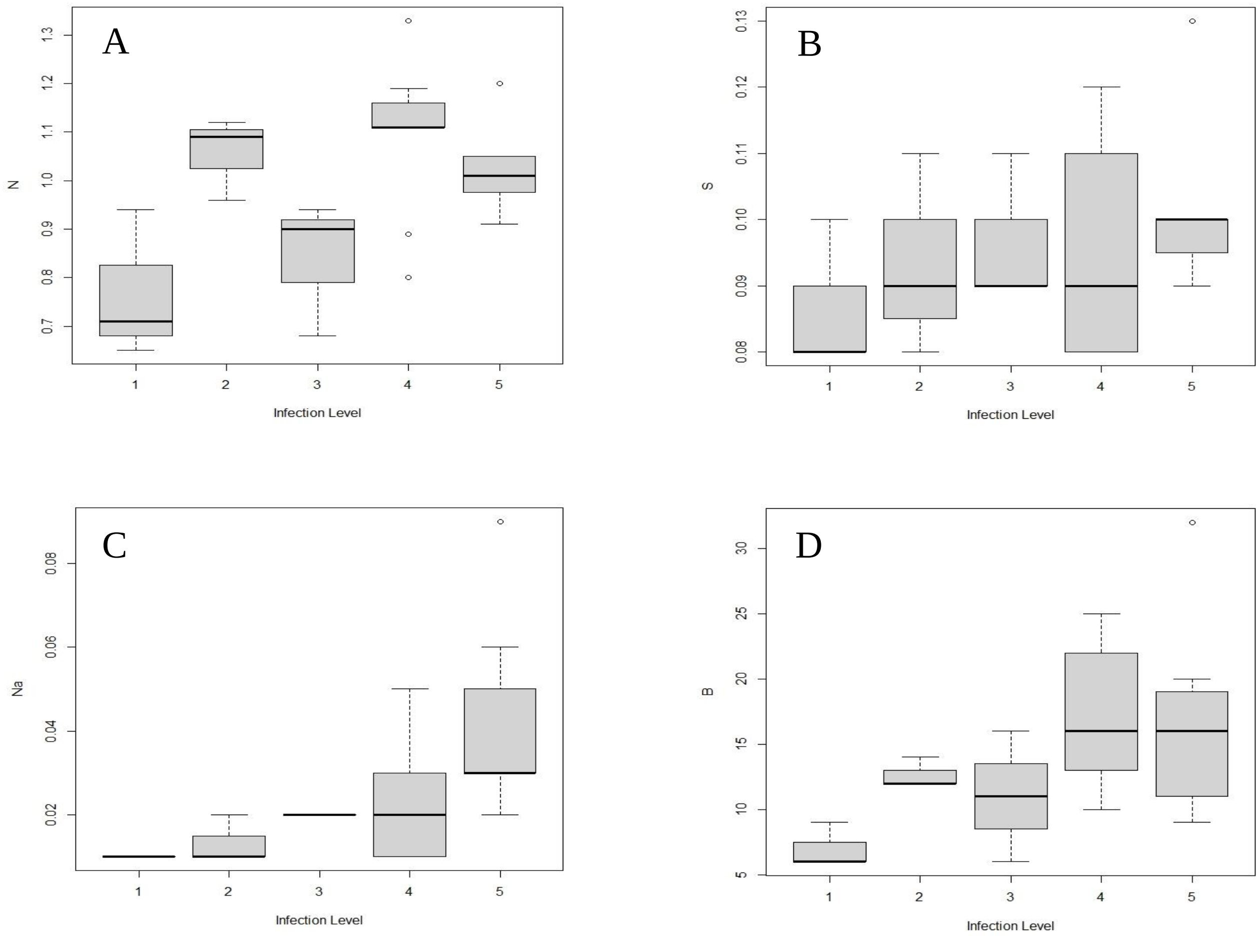
Materials & Methods

Flow-chart: Total phenolic extraction process



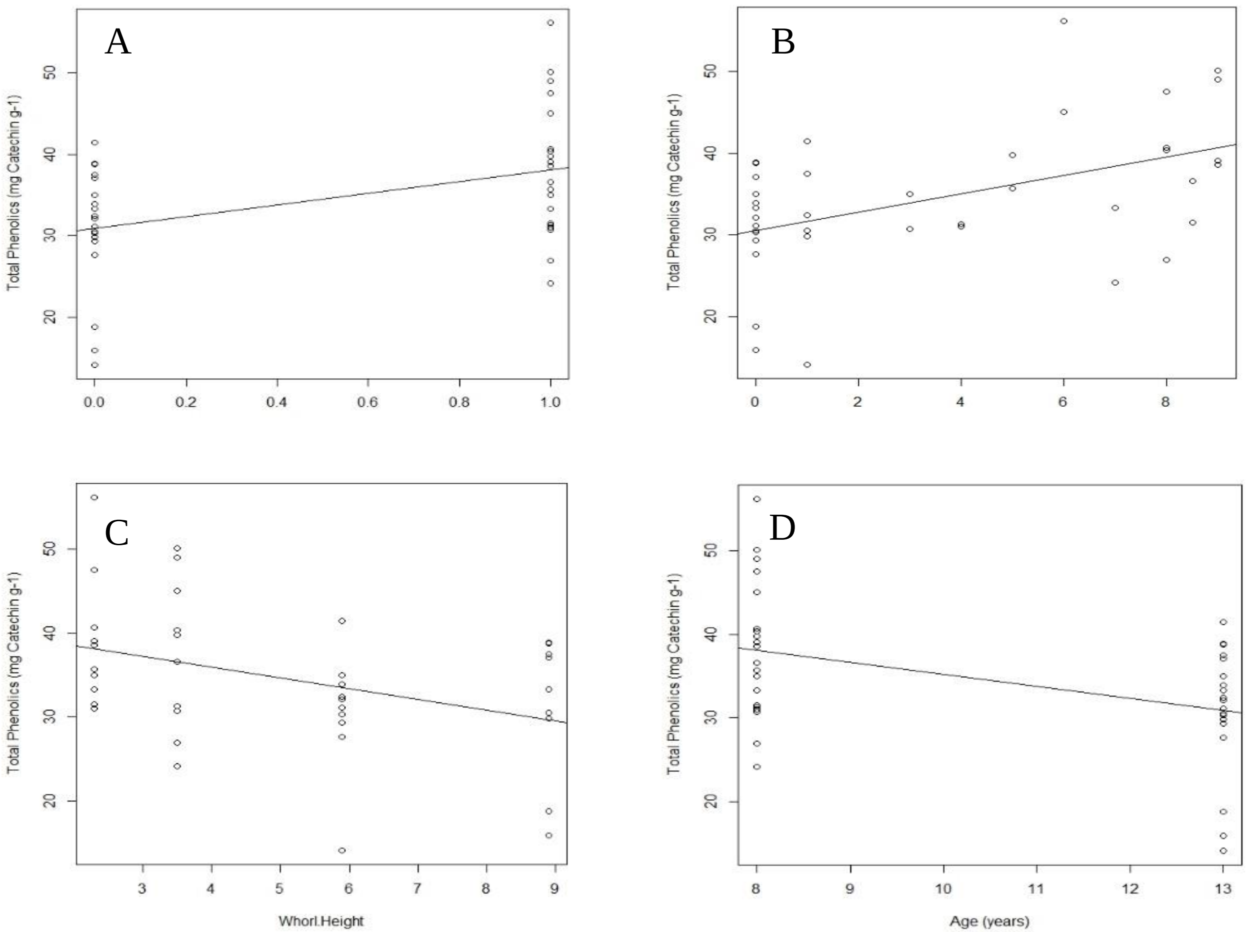
Statistical analyses were performed in R version 4.0.3 software

Results



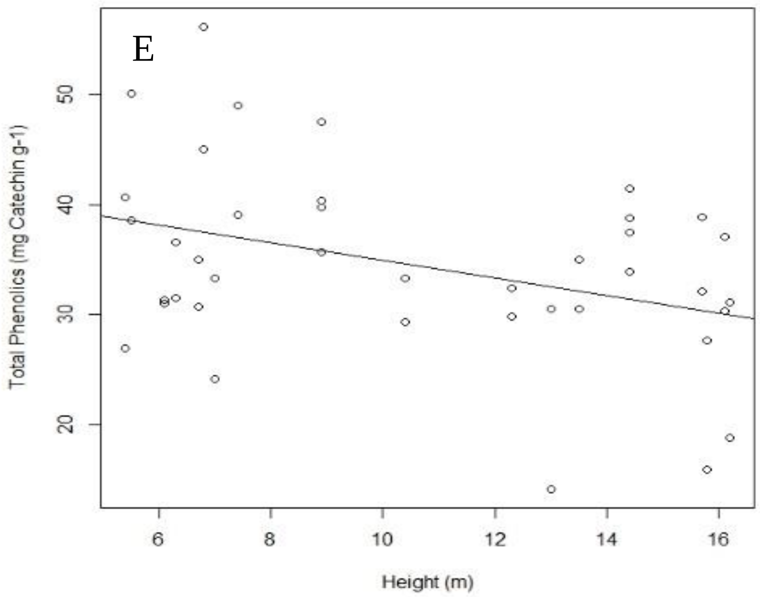
Response of foliar macro and micronutrients such as (A) Nitrogen (B) Sulfur (C) Sodium & (D) Boron with BSNB severity

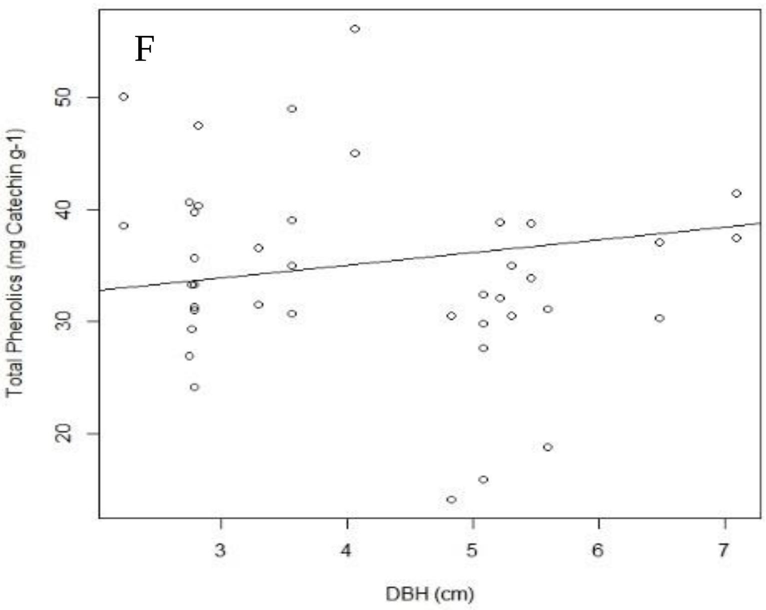
Results



Relationships between total phenolics concentration and (A) infection level (B) disease severity (C) whorl height (D) age

Results





Relationships between total phenolics concentration and (E) Height & (F) DBH

Conclusions

Loblolly pine foliage and foliar chemistry

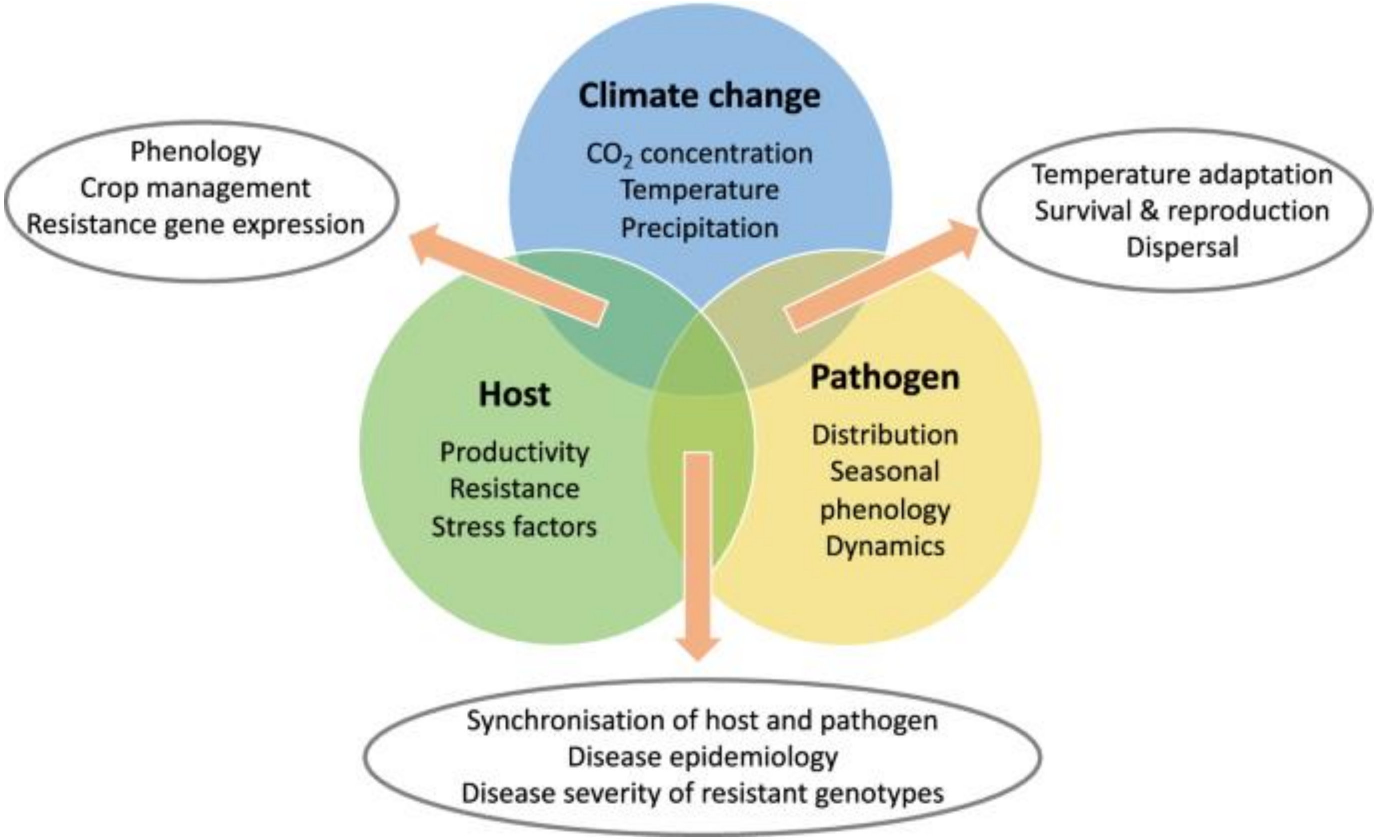
- Nitrogen (N), Sodium (Na), Boron (B) and Sulfur (S) positively correlated
- No correlation of other nutrient contents
- High variations in the needles
- Total phenolics increased

Lecanosticta acicola infection

- Foliar fungi can be influenced by foliar nutrients
- N, Na, B and S fertilization could increase disease severity
- Total phenolics production as a normal defense system
- High concentration could increase severity

Chapter V

Prediction of Loblolly Pine Defoliation Severity Associated with Changes in Pathogen Pressure in response to Climate Change in the Southeastern U.S.



Introduction

Needle disease and host susceptibility

- Climatic factors (temperature and moisture)
- Distribution and potential migrations of pathogens
- Spore survival and reproduction

Challenges

- Predictions of pathogen behavior
- High degree of uncertainty
- Long-lived nature of trees vs short life-span of pathogens

Loblolly pine defoliation and tree mortality

- Increased pathogen pressure
- Host susceptibility

Objectives

To determine climatic patterns that might drive the emergence and spread of loblolly pine defoliation

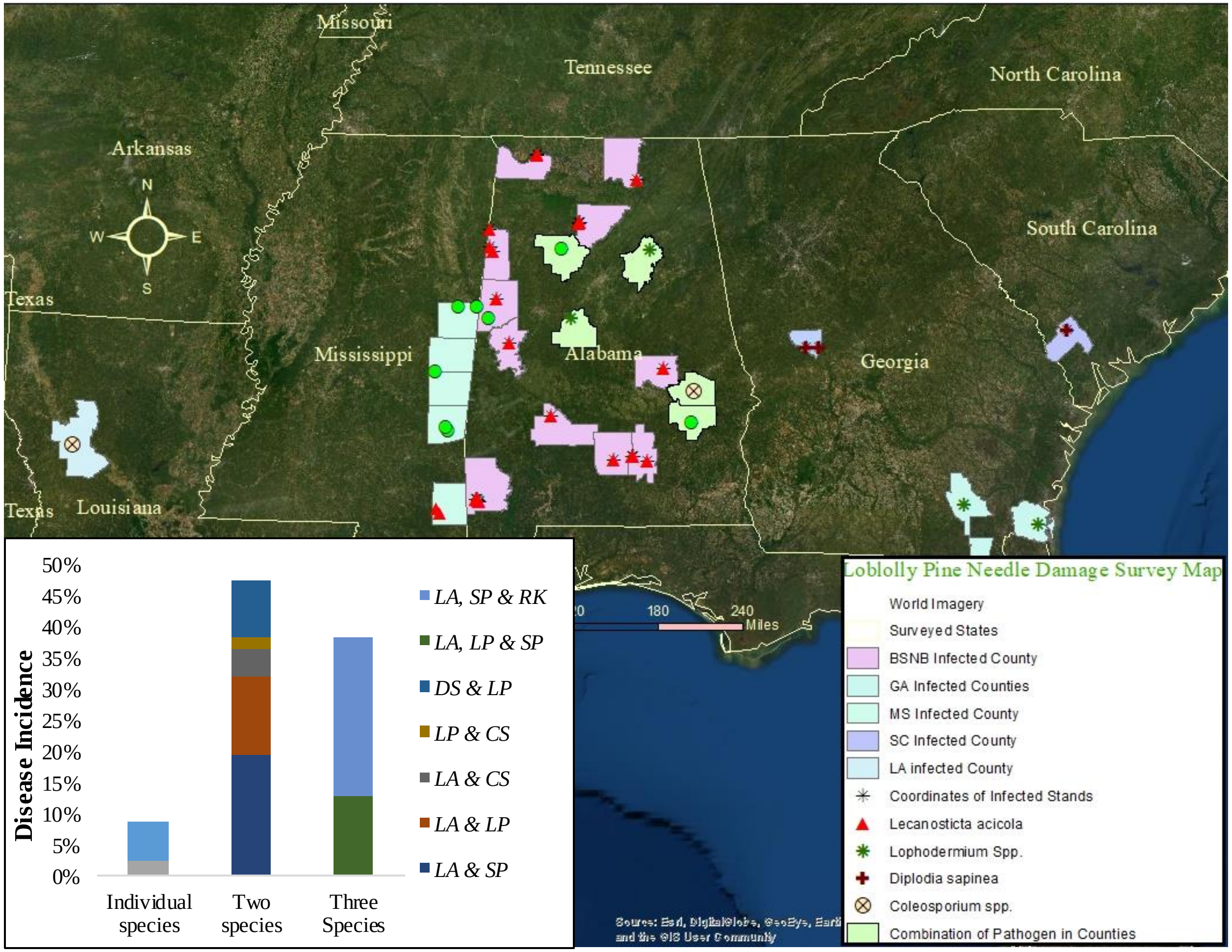
To develop climatic regression models to predict defoliation severity in following years to aid private landowners, forest managers to adjust their management strategies accordingly

Materials & Methods

Visual rating and map of pathogen distribution:

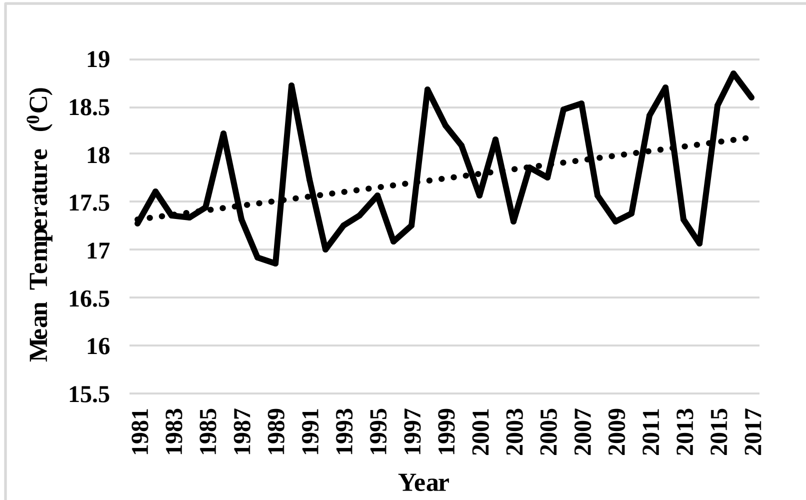
- Data used from chapter I
- Ten miles radium and data availability
- Preceding year climate variables
- NOAA online data
    - Maximum and minimum temperature
    - Sum of precipitation
    - Relative humidity
- Forty-nine variables
- Stepwise regression
- SAS 9.4 version software

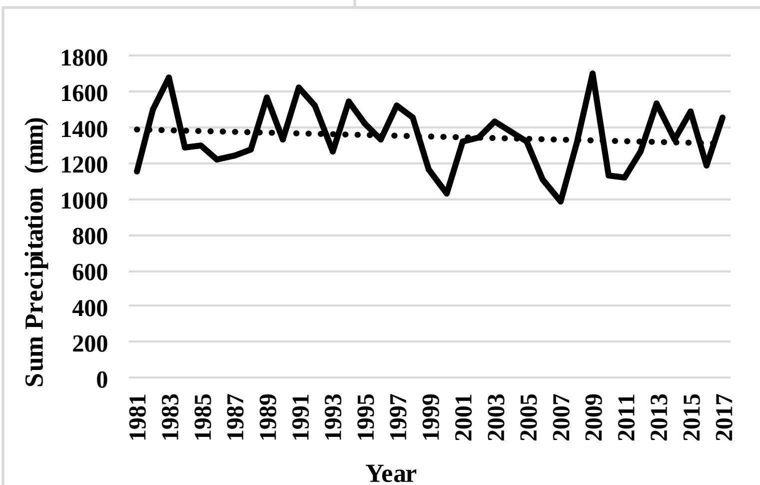
Needle Pathogen Distribution

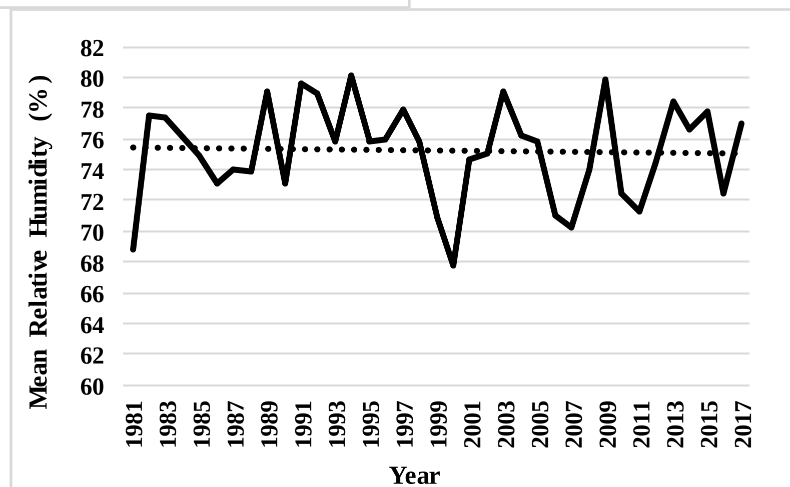


Pathogen distribution map of *Lecanosticta acicola* (LA), *Coleosporium* spp. (CS), *Lophodermium* spp. (LP), *Sydowia polyspora* (SP), *Rhizosphaera kalkhoffii* (RK) and *Diplodia sapinea* (DS)

Long-term Regional Weather Data







Mean temperature, sum of precipitation and mean relative humidity during loblolly pine growing season at 19 infected sites in the southeastern United States

Climatic Regression Model

Model	Variables	Model Prob. > F	Adjusted R^2
1-factor	Min. May T	0.0413	0.2277
2-factor	Modified Spring P, Fall P	0.0010	0.6298
3-factor	Min. April T, Modified Spring P, Fall P	0.0002	0.7725
4-factor	Min. April T, Max. July T, Modified Spring P, Fall P	<.0001	0.8809
5-factor	Min. April T, Max. July T, April P, Modified Spring P, Fall P	<.0001	0.9121

Conclusions

Based on climatic regression models

- Eleven climatic variables identified
- Five variables correlated
- April, May and July temperature
- April, May, June, July and August precipitation

Current emergence of LPND

- High-density occurrence of loblolly pines
- Proximity of water body
- Poor drainage
- Wide geographic distribution
- Increasing temperature and precipitation

Future directions of the study

Management perspectives

- Establishment of inoculation protocol
- Resistant loblolly pine families
- Biomarker development

Research perspectives

- Population genetic study
- Whole genome sequence
- Long-term monitoring plots

Finally, LPNDM must be monitored, forecasted, planned and taken into management strategies

Acknowledgements

Committee members

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Molecular Mycology Lab
USDA Forest Service Lab
Waypoint Analytical Lab
Forest Products Lab

Forest Health State Cooperative

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