Identification and Distribution of Fungal Pathogens associated with Loblolly Pine Defoliation and Mortality in the SE US

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Brown Spot Needle Blight Assessment Workshop

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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

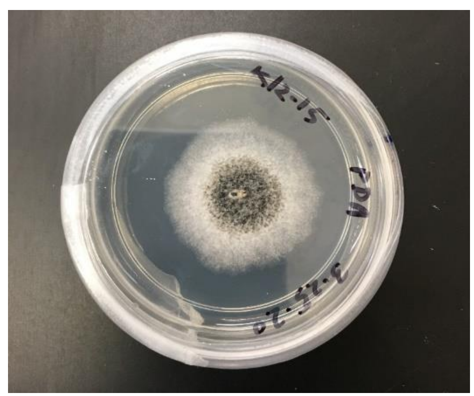
For example,

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

Phytophthora needle disease in Chile

Phytophthora needle disease in New Zealand

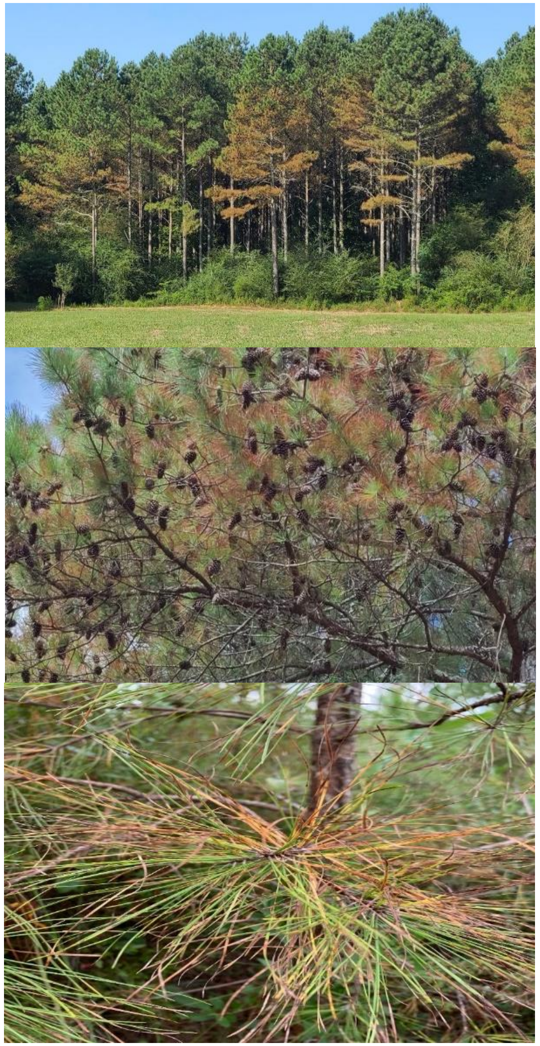
Isolation and Identifying of Fungi associated with Loblolly Pine Needle Damage and Mortality



Introduction

Loblolly pine defoliation and tree mortality

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

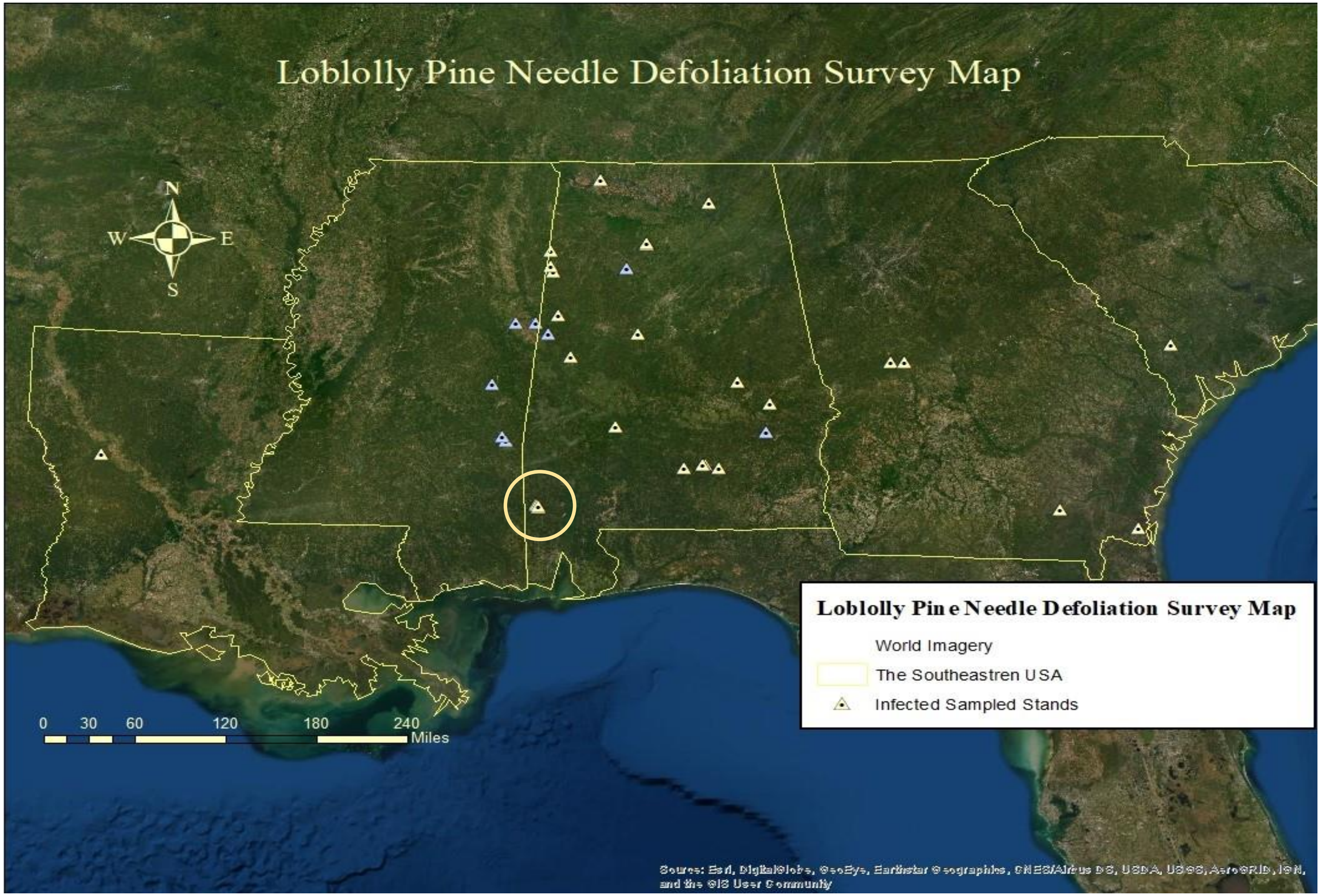


Objectives

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



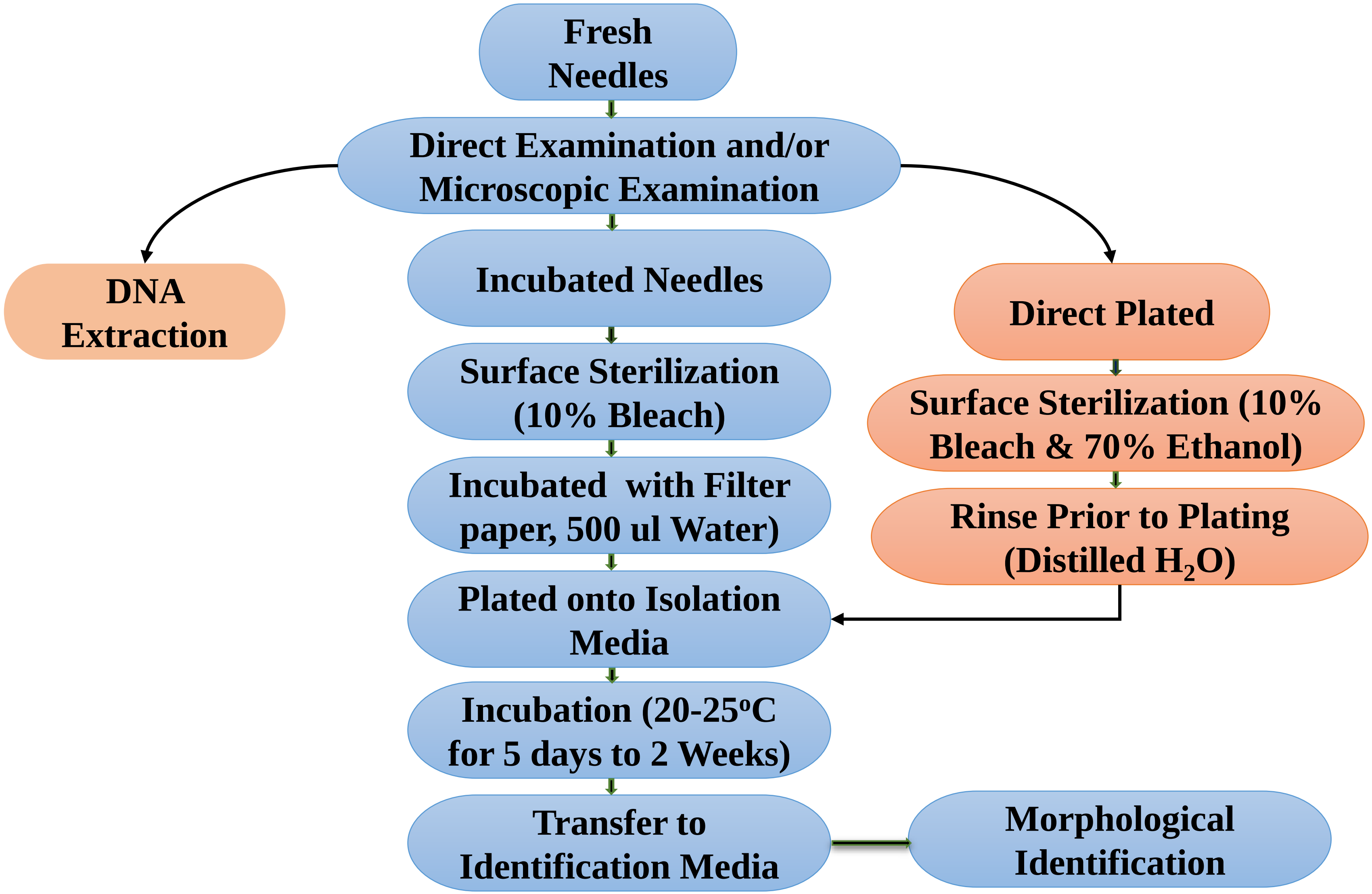
Map showing permanent plots in AL and surveyed stands in SC, GA, AL, MS, and LA from 2019 to 2021

Study Area

Sporulation period of five needle pathogens

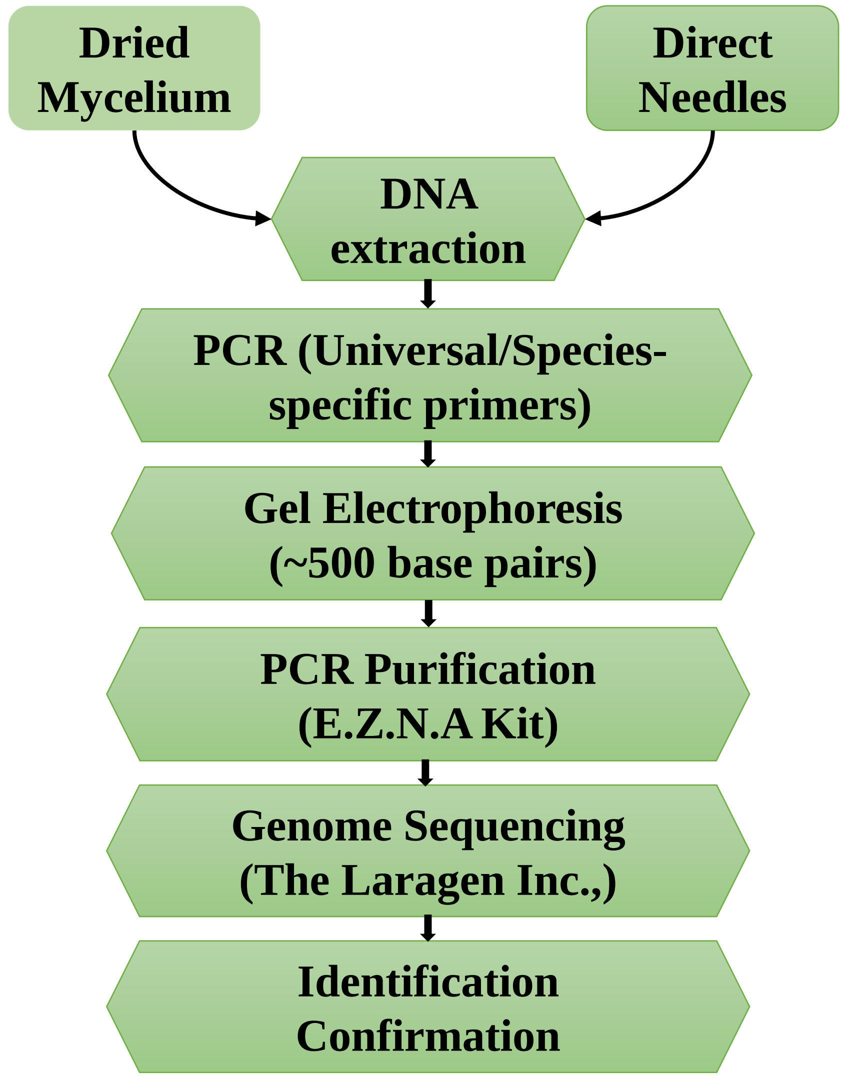
Needle Disease	Causal Agent	Host	Sporulation Period
Phytophthora needle blight	*Phytophthora* spp.	Pines, Oak, Douglas fir,	May to November
Brown spot needle blight	*Lecanosticta acicola*	Over 53 different pine species	March to October
Dothistroma needle blight	*D. septosporum* *D. Pini*	82 Pinus taxa	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



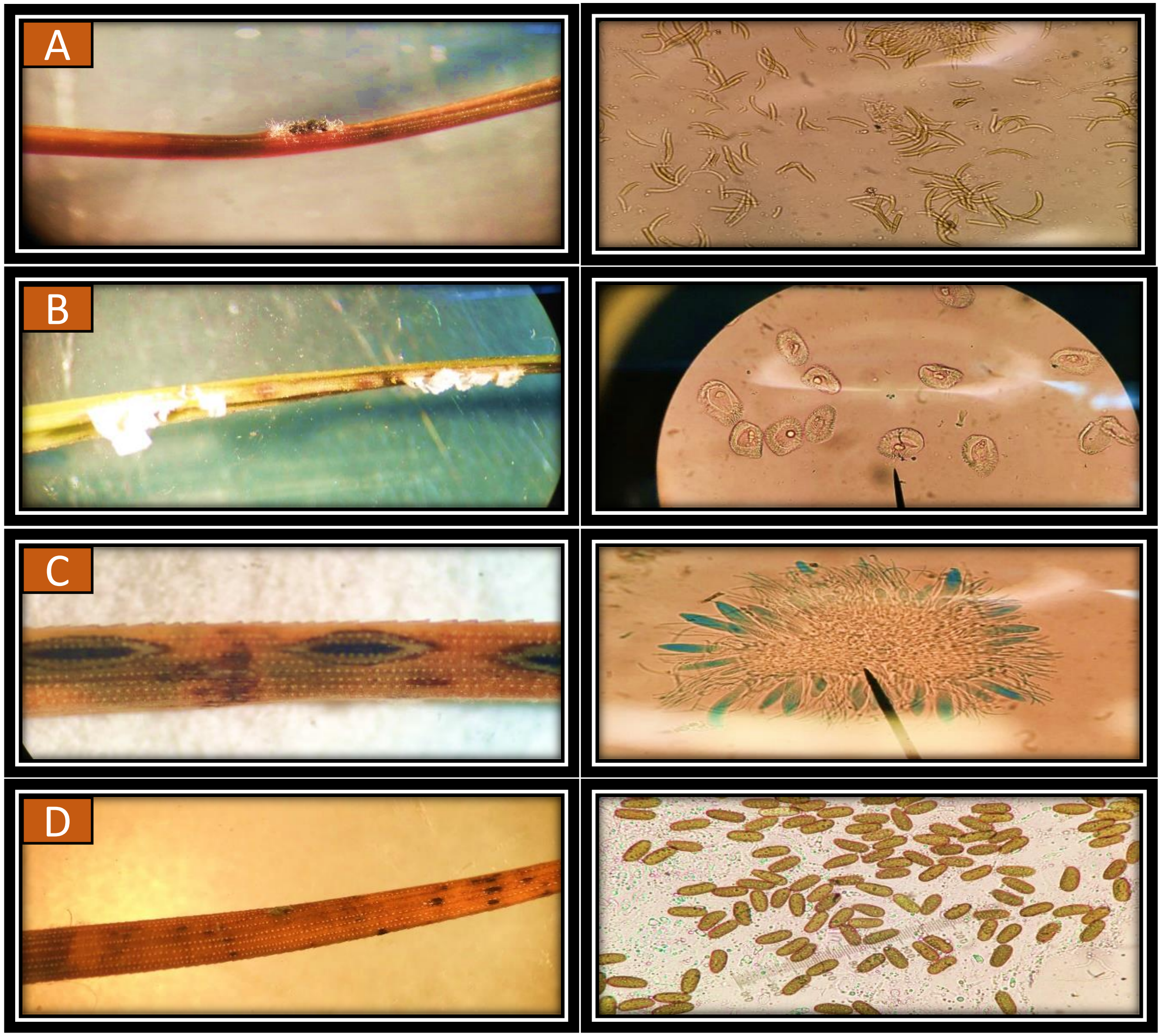
Cultural methods for fungi identification

Materials & Methods



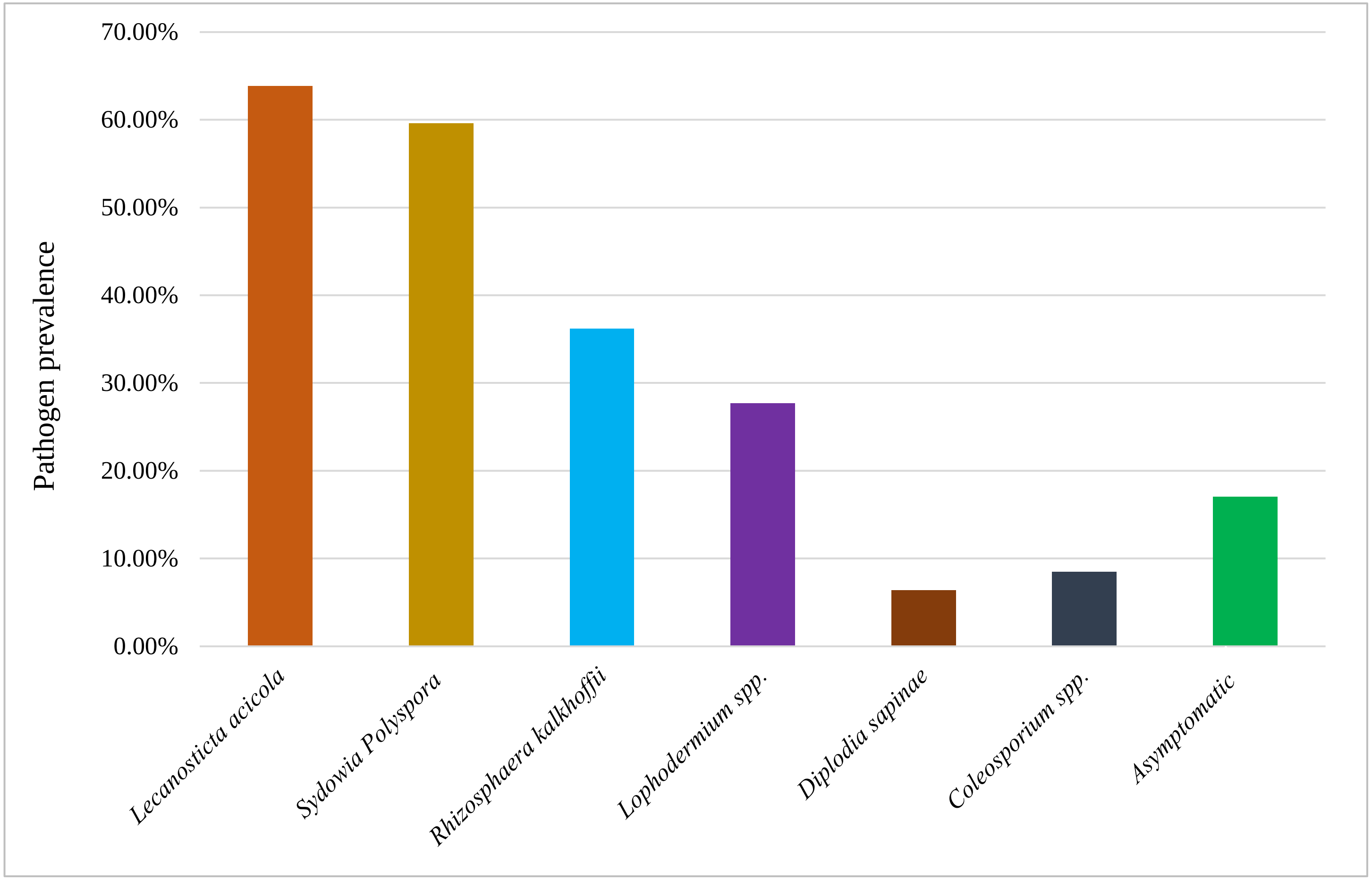
Molecular methods for fungi identification

Results



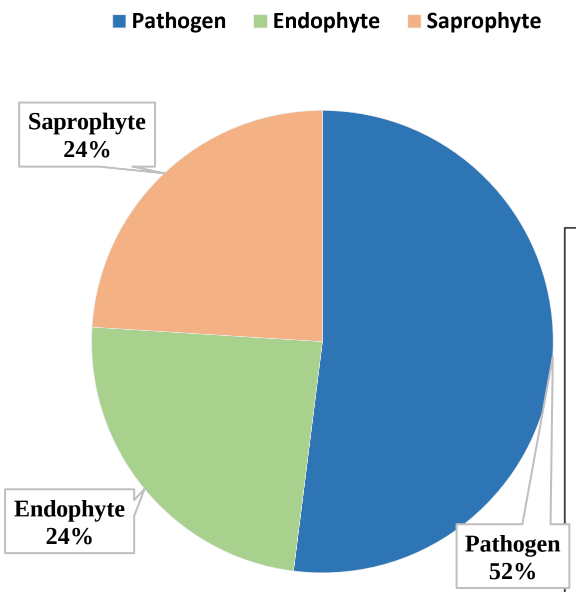
Sporulation Chamber: Disease symptoms and reproductive structures of (A) *L. acicola* (B) *Coleosporium sp.* (C) *Lophodermium sp.* & (D) *D. sapinea* on loblolly pine

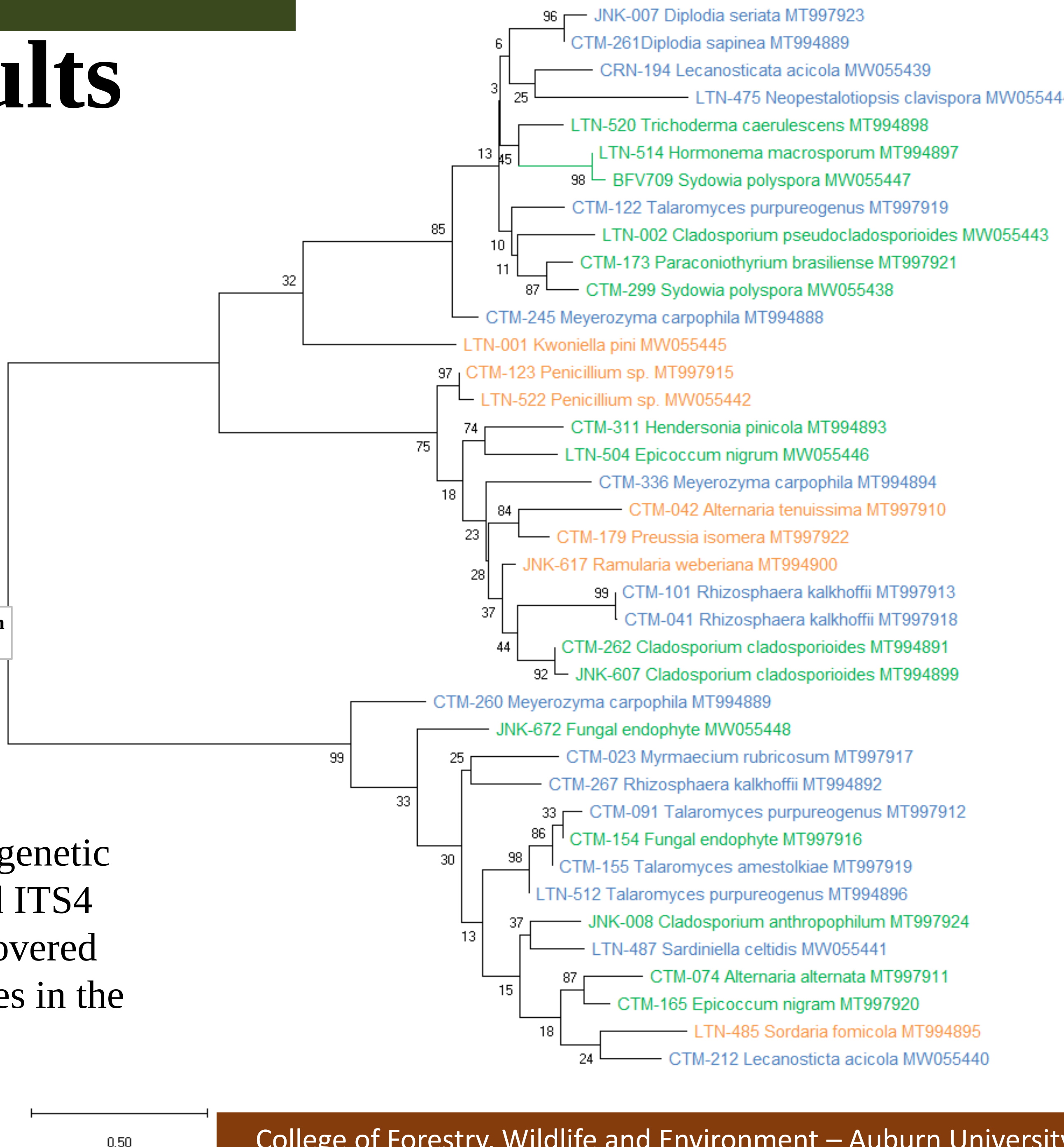
Results



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

Results

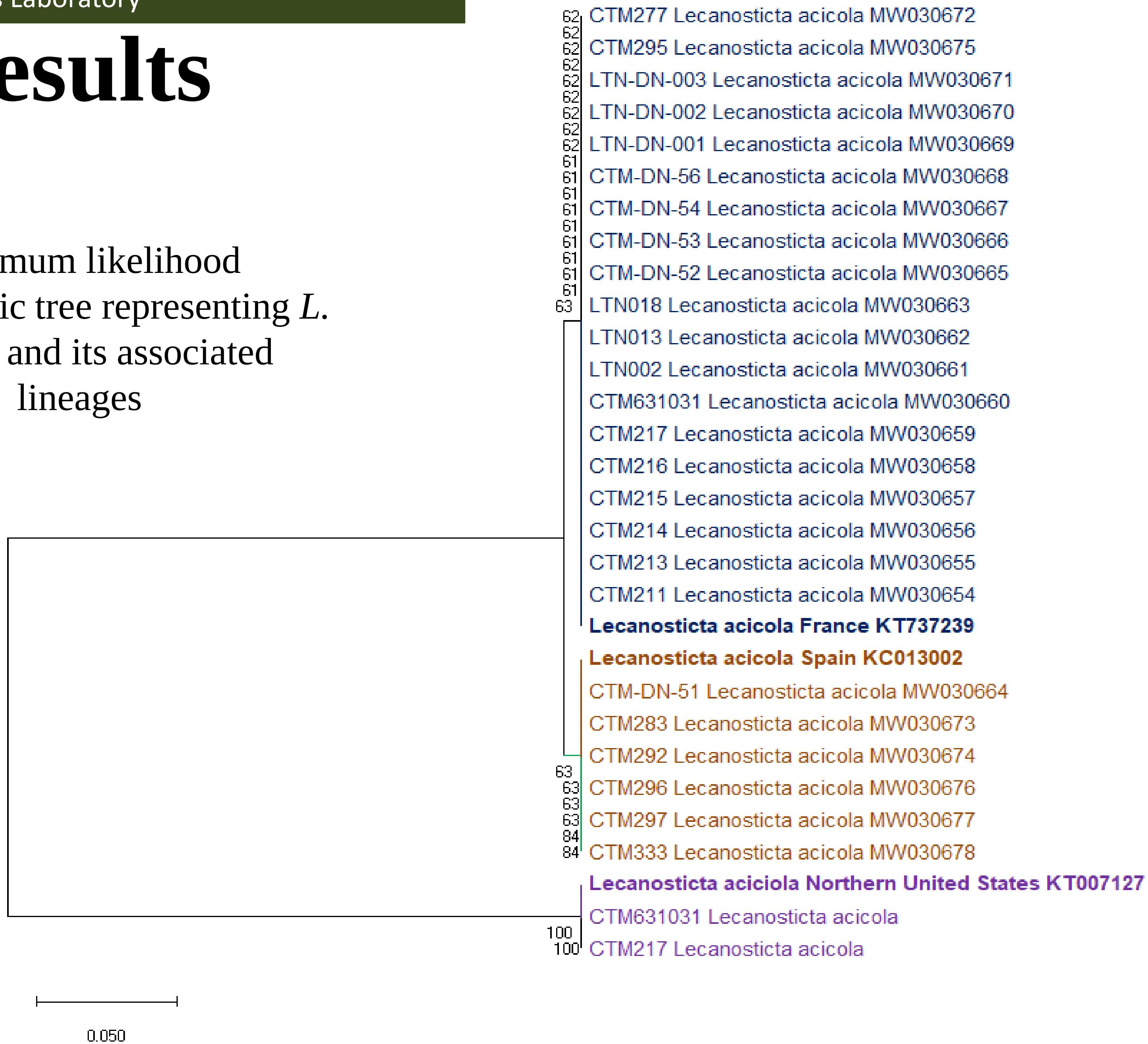




Neighbor-joining phylogenetic tree based on ITS1 and ITS4 sequences of fungi recovered from loblolly pine needles in the study

Results

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



Results

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

- Predominant pathogen found (except in GA)

- Three distinct lineages

- Asexual state recovered

- Positive for a single mating type loci, MAT-1-1

Conclusions

Lecanosticta acicola associated with loblolly pine defoliation and tree mortality

- AL, MS, LA, SC
- Less genetically diverse (only one mating type)

Sydowia polyspora

- Endophytic to pathogenic (?)
- Disease severity increased when present

Genetic and environmental factors

- Needs further investigation

Needle Pathogen, *Lecanosticta acicola*, effects on *Pinus taeda*

Needle and Shoot Lengths

Introduction

Needle pathogen, *Lecanosticta acicola*

- life cycle on needles

- Overwinters

(a) Vegetative mycelium

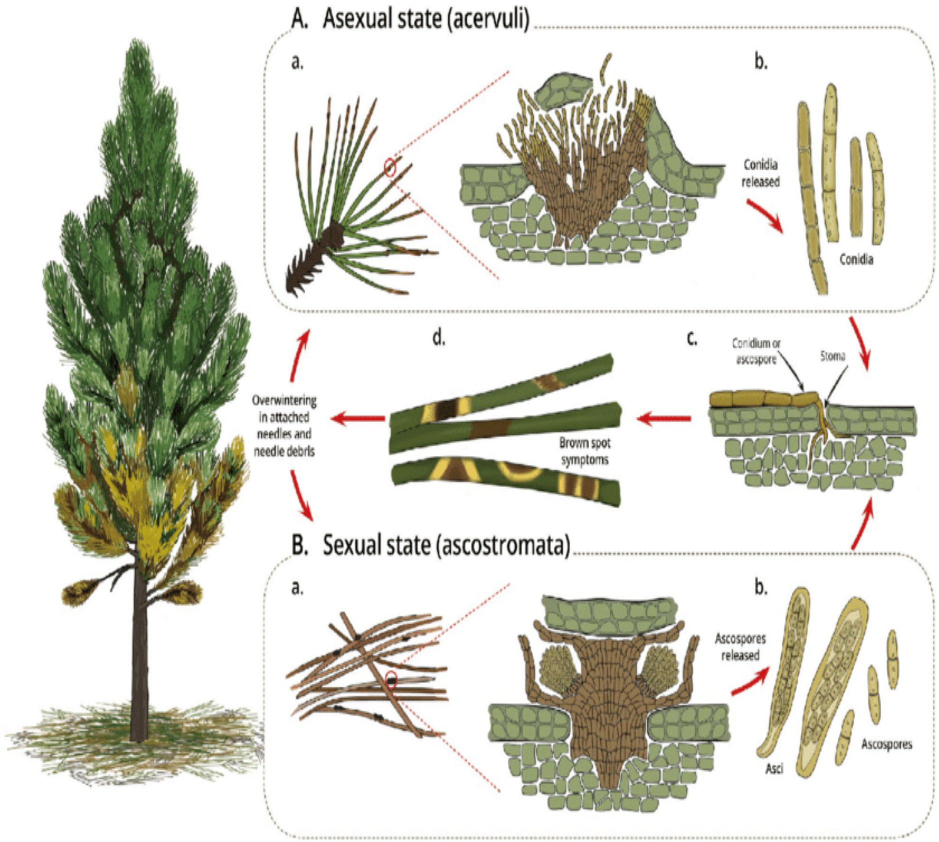
(b) Asexual acervuli

(c) Sexual ascostromata

- Light, temperature and humidity

- Conidia and ascocspores

- Air-currents or rain-splash spores



Life Cycle of the brown spot needle blight fungus, *Lecanosticta acicola*

Introduction

Healthy trees

- Robust shoot growth, long and lush green needles

- Optimal growth and productions

Shoot development in pine trees

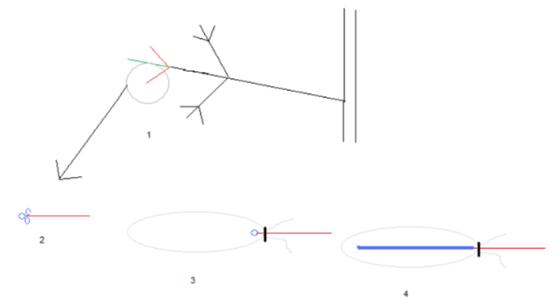
- Long shoot and short shoot development

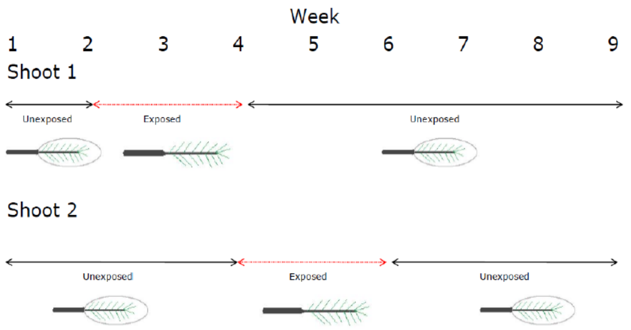
- Impacts of stress and growth promotion factors

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

- Needle and shoot sizes have not been assessed

- Disease progression





Objectives

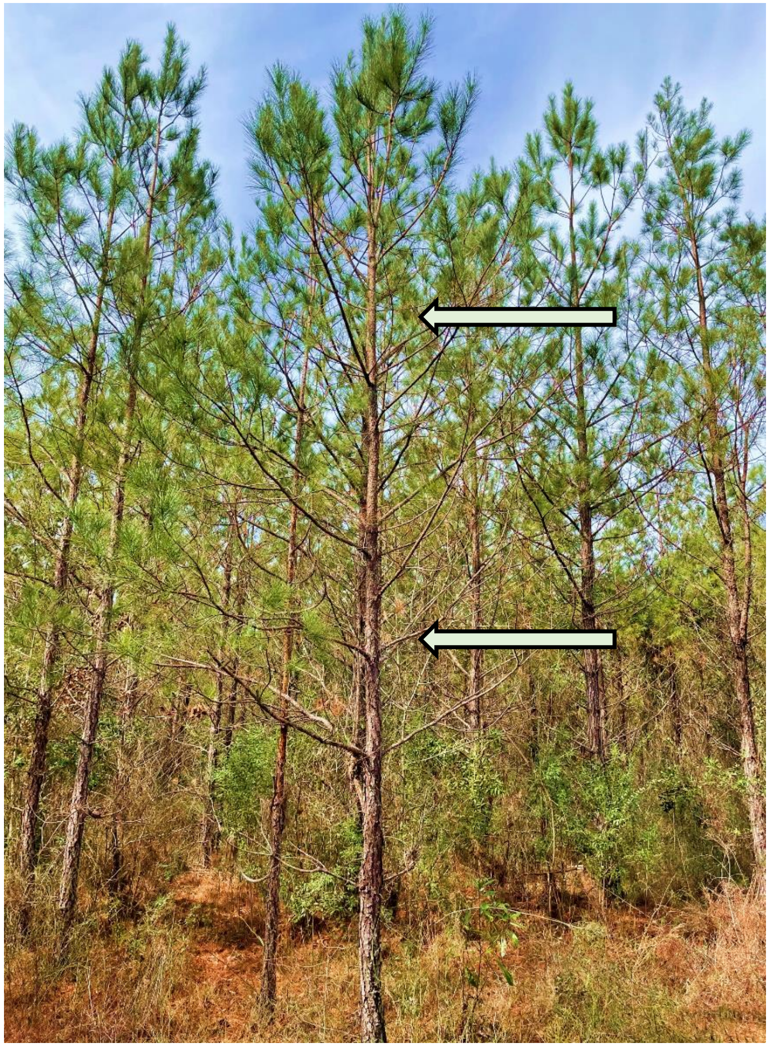
To assess brown spot needle blight fungus, *Lecanosticta acicola* effects on shoot and needle lengths

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

Materials & Methods

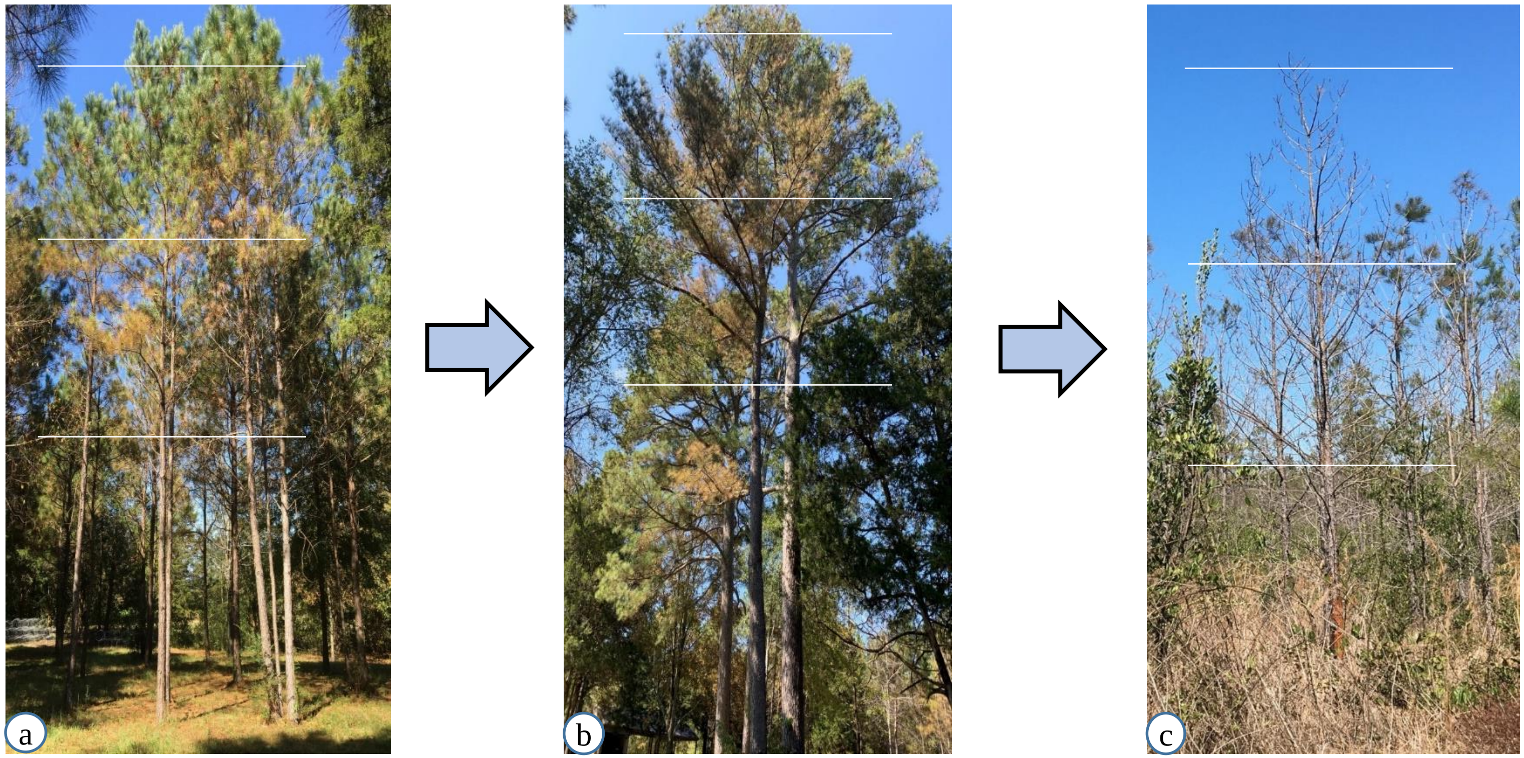
Study design and data collection:

- Two infection levels
- Two whorl heights
- 28 low incidence and 33 high incidence trees
- 10 fascicles
- End of the growing season
- 2019 and 2020



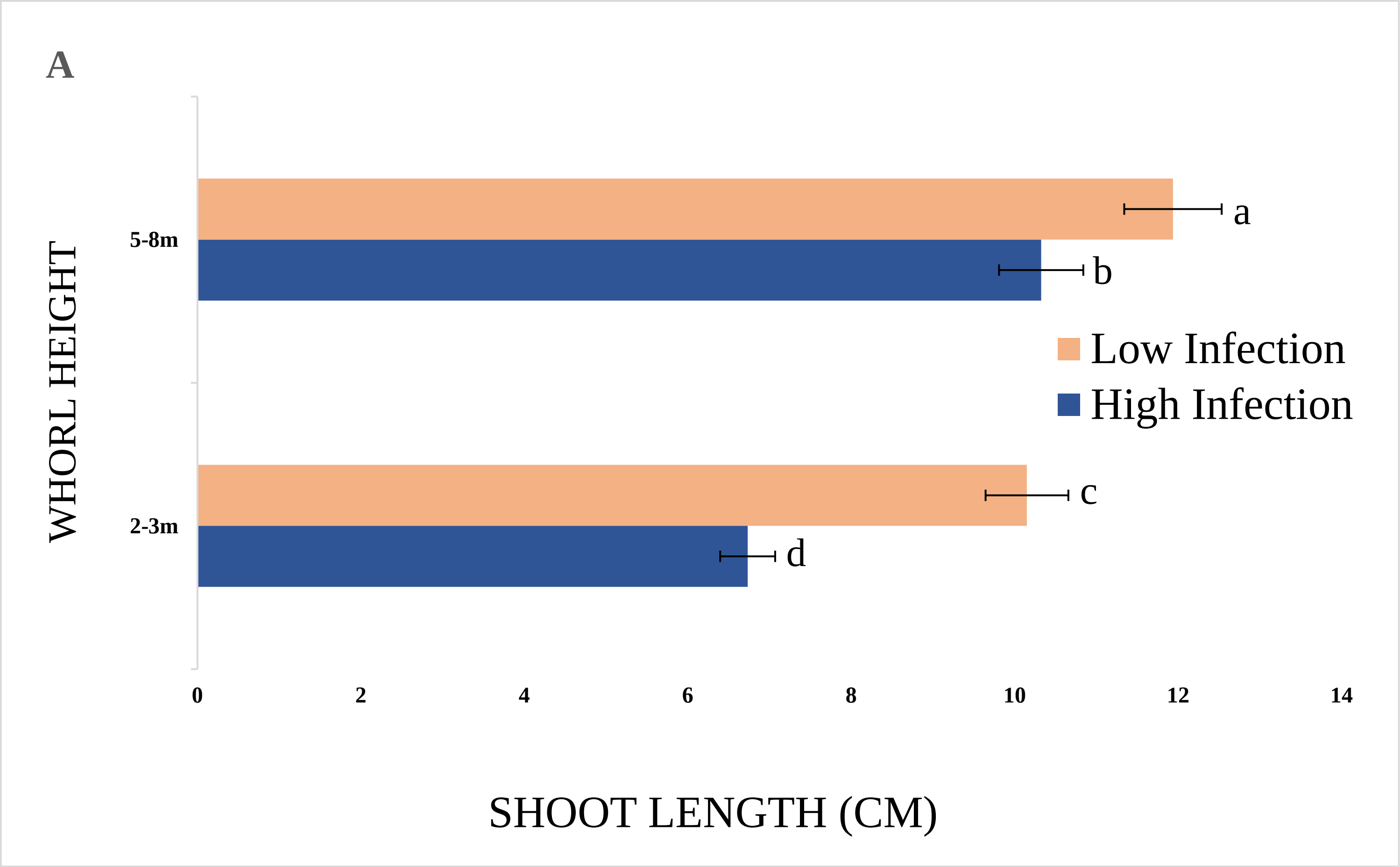
Loblolly Pine Health Monitoring

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



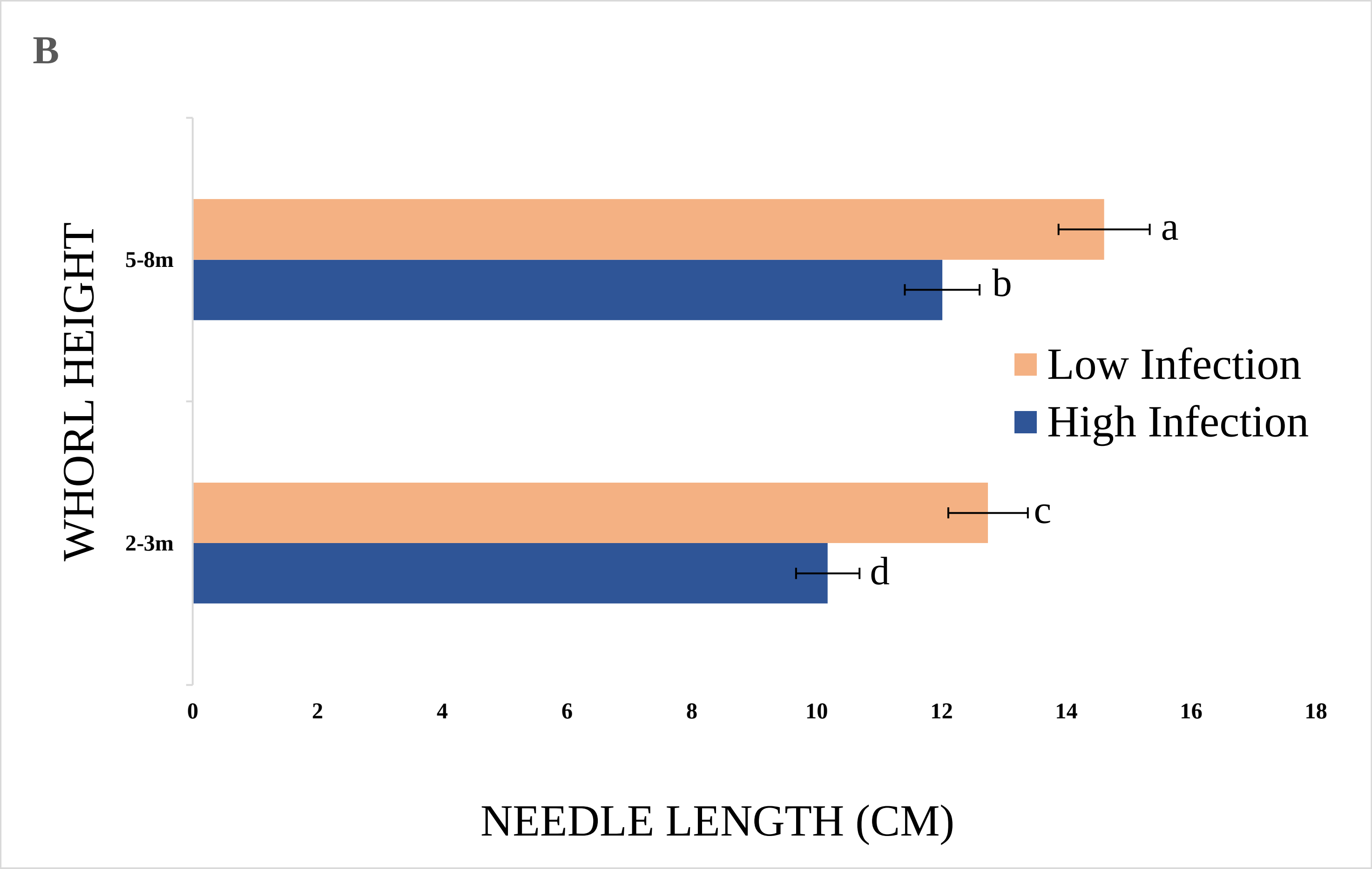
Data were analyzed using MS Excel 2010. Response variable was either "chlorosis rating per tree" or "defoliation per tree"

Results



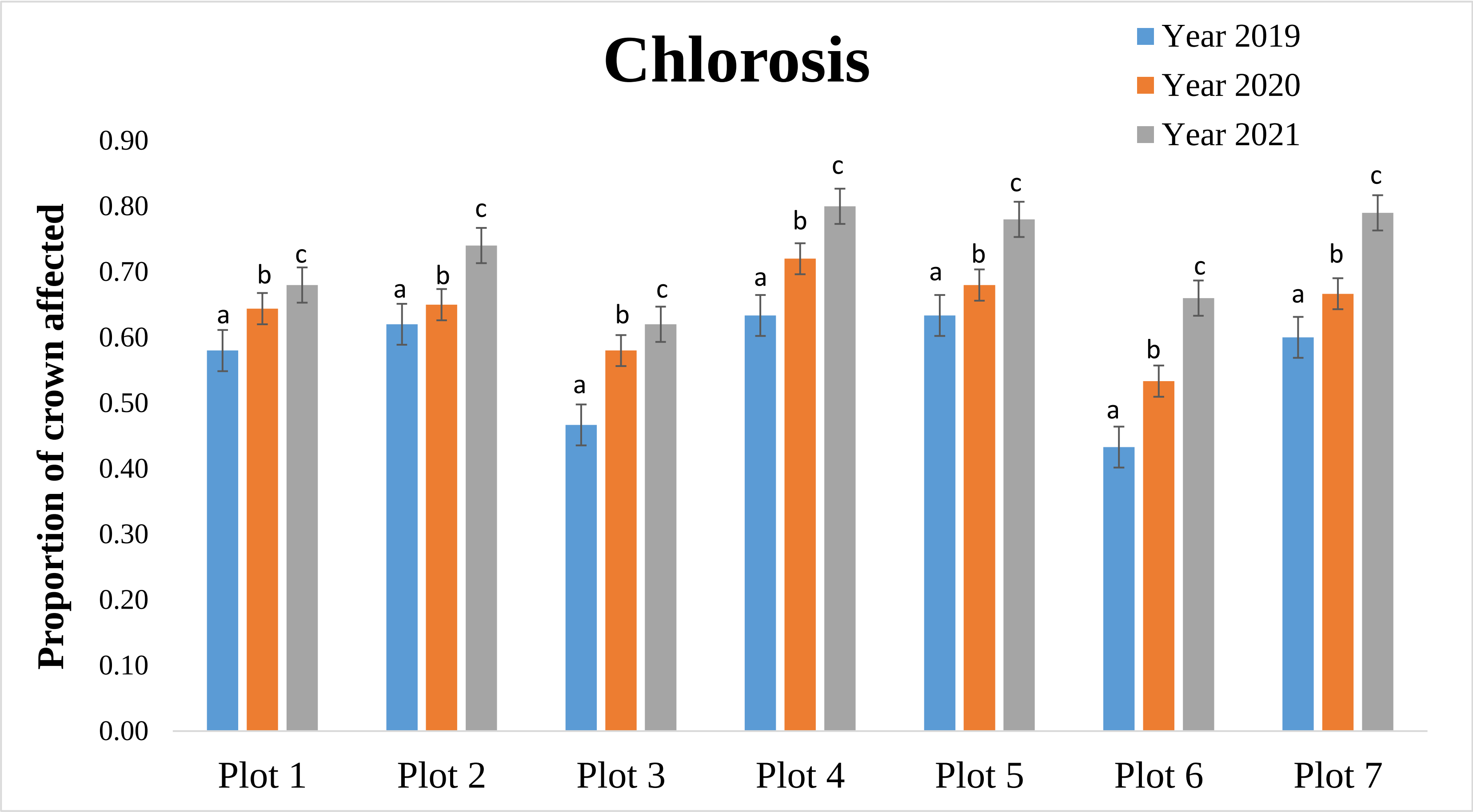
Observed means and standard errors of shoot length

Results



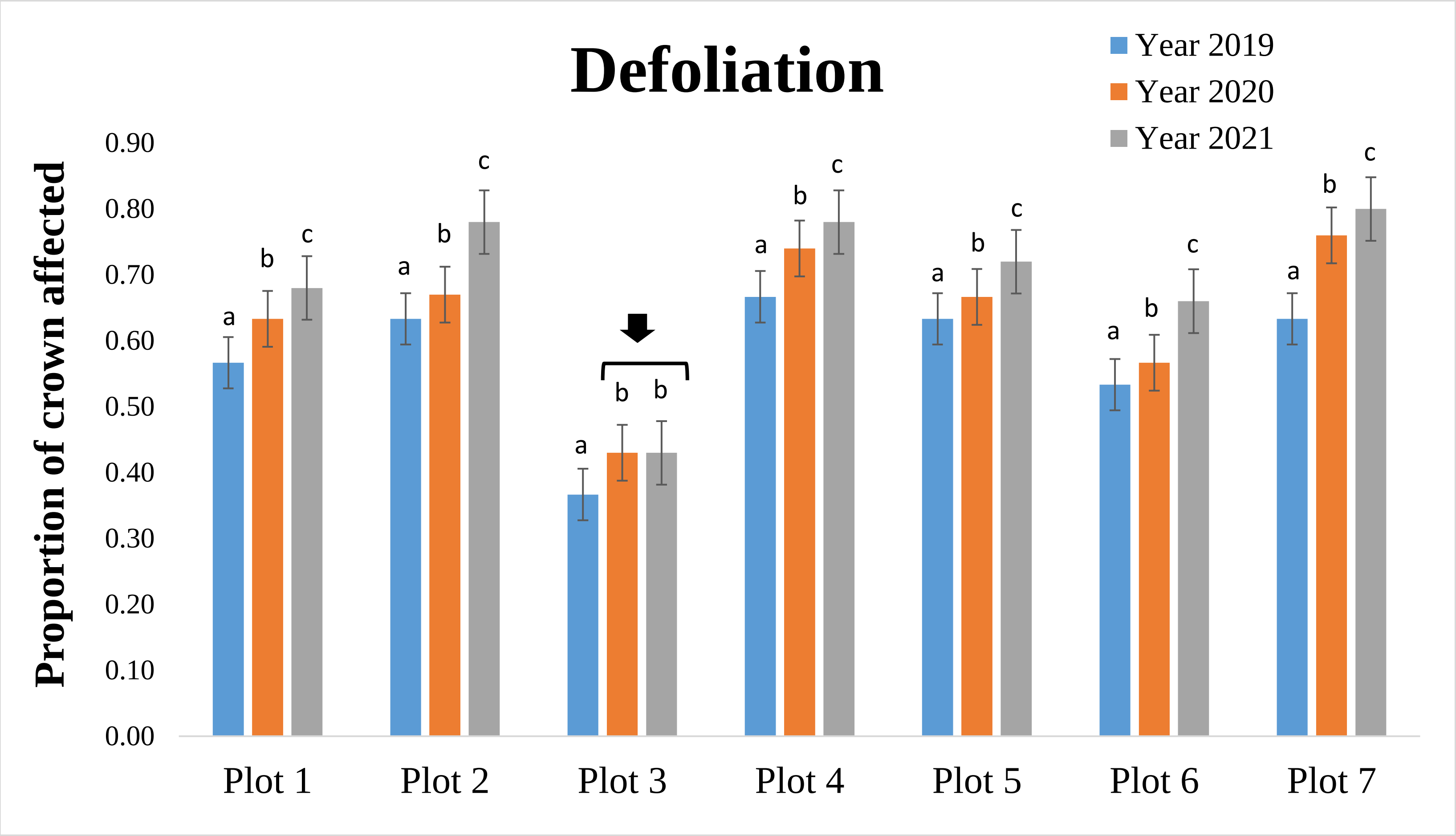
Observed means and standard errors of needle length

Results



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

Results



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

Results

Needle pathogen, *L. acicola*

- Healthy trees becoming infected
- Unhealthy trees becoming more chlorotic and defoliated
- Lower crown to upper crown
- Premature mortality of the trees

Repeated *L. acicola* infection results in

- Significantly shorter needles
- Significantly shorter shoots
- Correlated to whorl height

Conclusions

Needle and shoot length reduction

- Chlorosis, necrosis and premature defoliation
- Photosynthesizing area and reserves
- Carbon deficit
- More infection on lower crown
- Genetic effects

***Lecanosticta acicola* infection**

- Shoot and needle lengths reduced
- Whorl height and shoot and needle lengths reduced
- Altered leaf mechanical support and physiological functions

Lecanosticta acicola impacts Nutrient Content and Total Phenolics in *Pinus 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

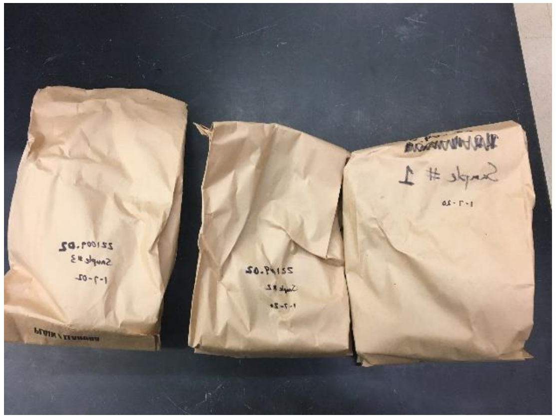
To determine the relationship between foliar nutrients and infection level to see how nutrients interact with *Lecanosticta acicola* severity

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

Materials & Methods

Foliar Nutrient Content Analyses:

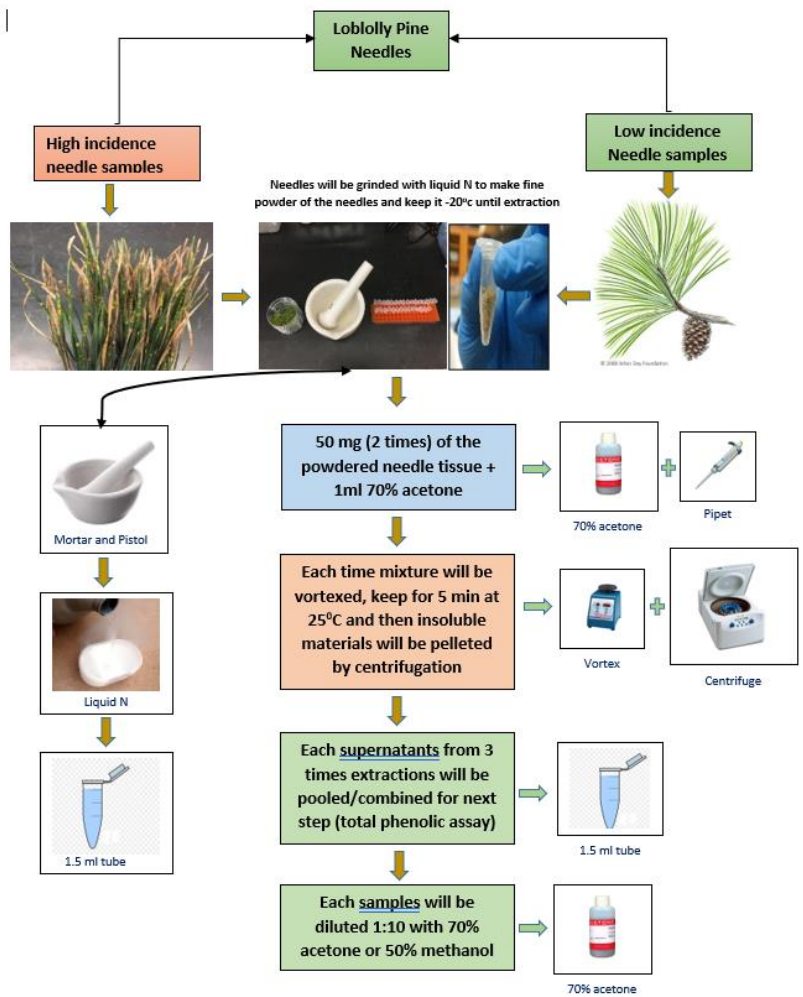
- Seven experimental plots
- Destructive sampling of foliage
- A 0.22 mag caliber rifle
- Fifty trees sampled
- Fifty fascicles per tree
- Oven-dried at 70°C
- 0.5 mm mesh screen
- Waypoint Analytical Laboratory



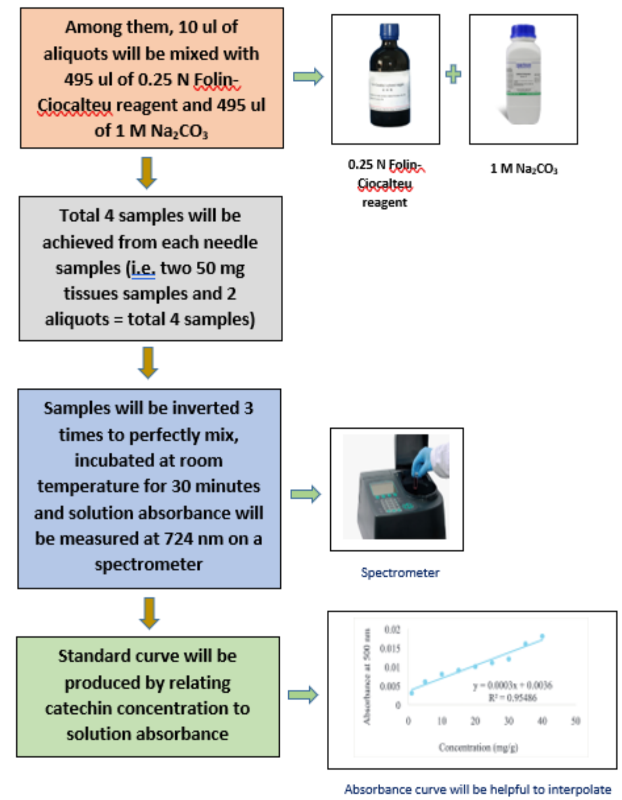
Materials & Methods

Total Phenolics Analyses: (collected from low and high incidence plots)

Flow-chart: Total phenolic extraction process

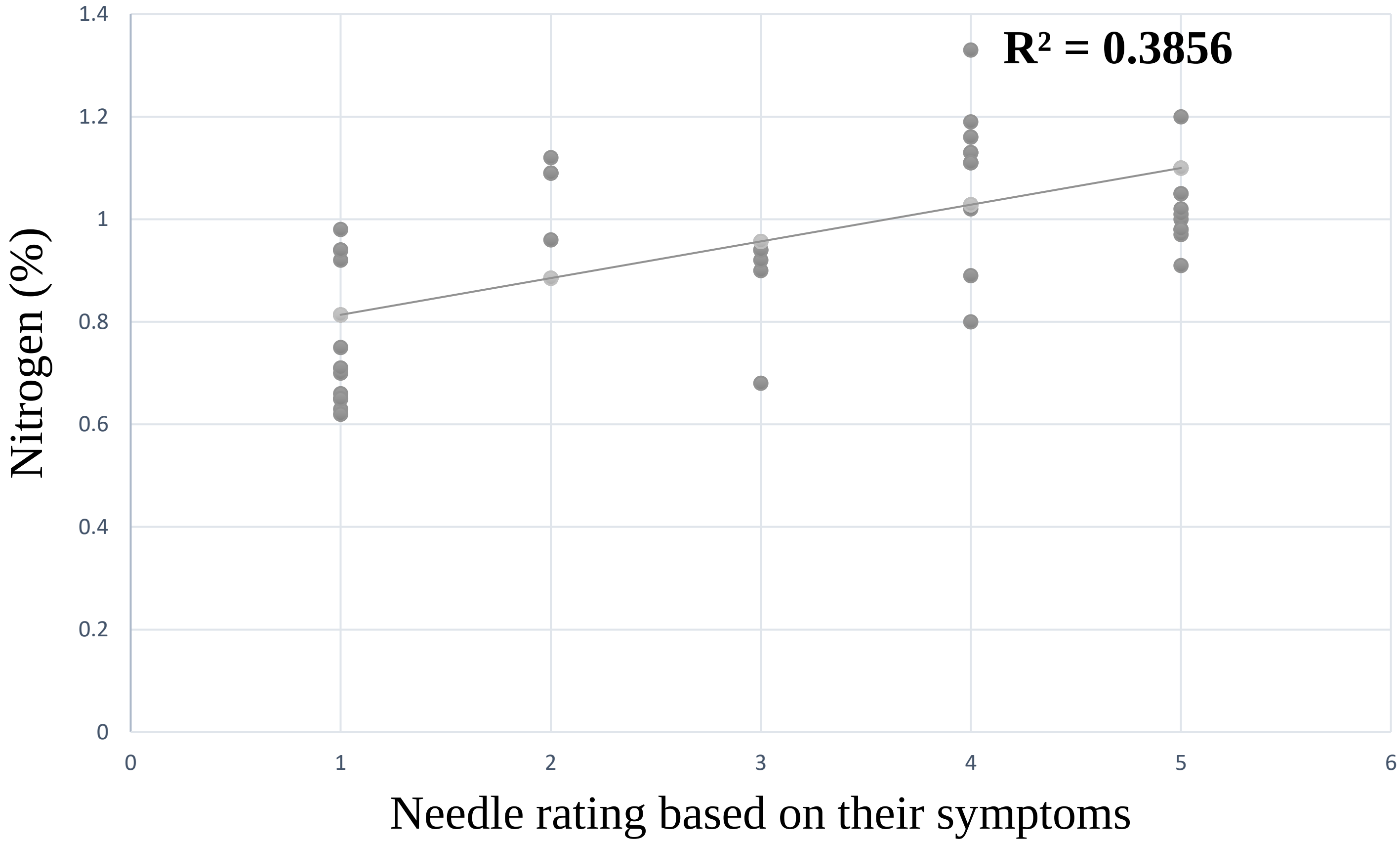


Flow-chart: Total phenolic extraction process (follow-up)



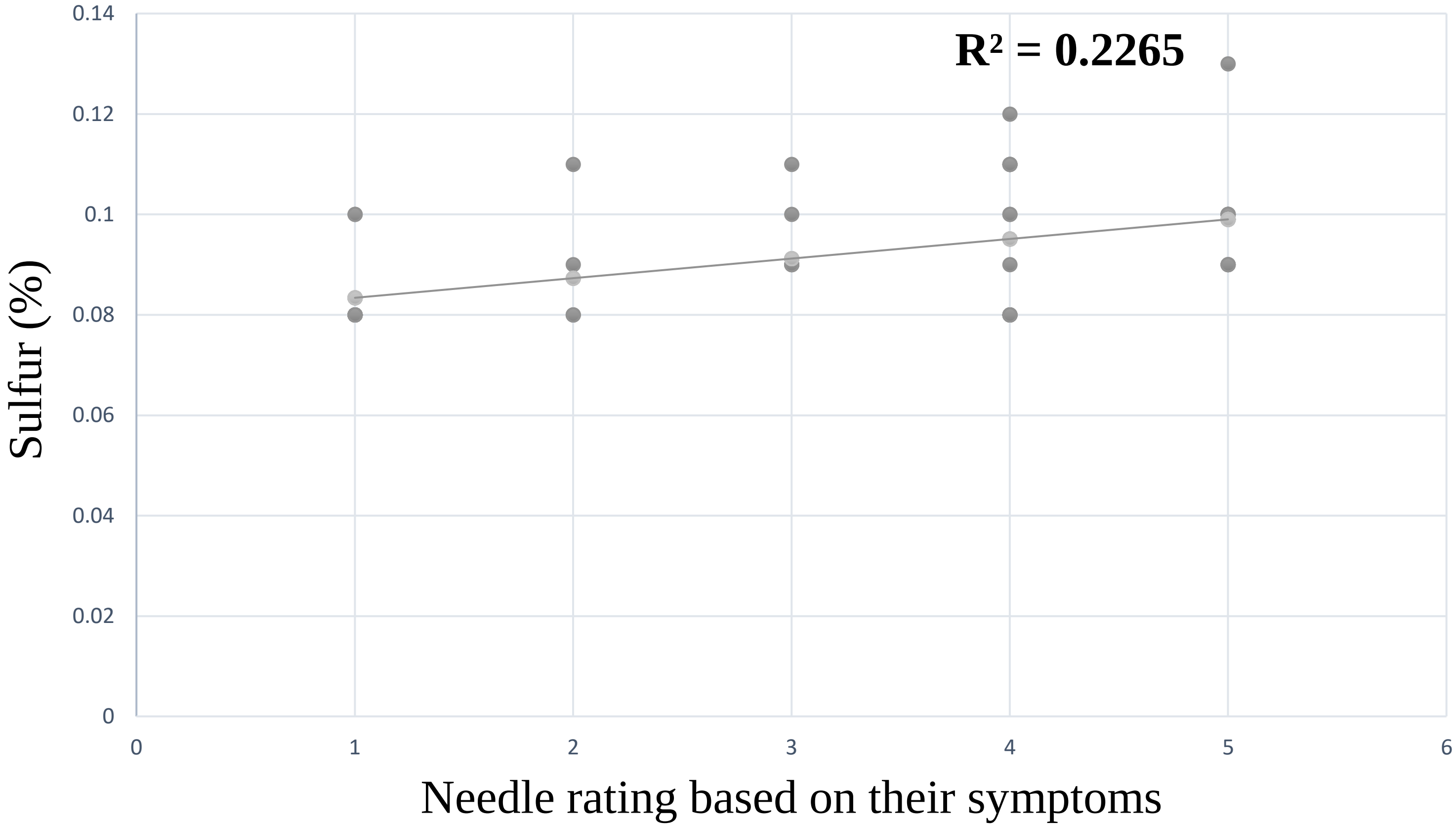
Statistical analyses were performed in R version 4.0.3 software

Results



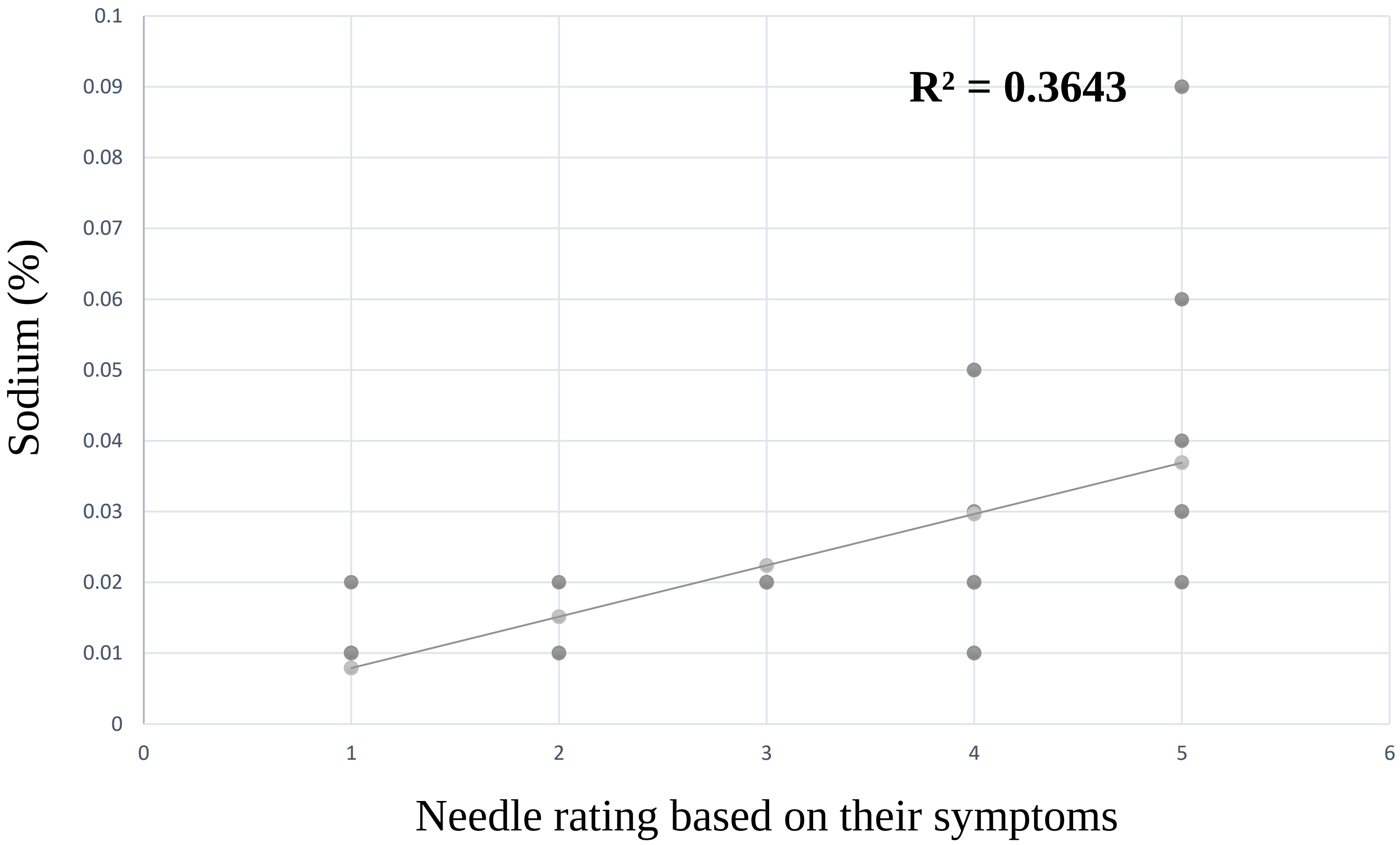
Relationships of Nitrogen with BSNB severity

Results



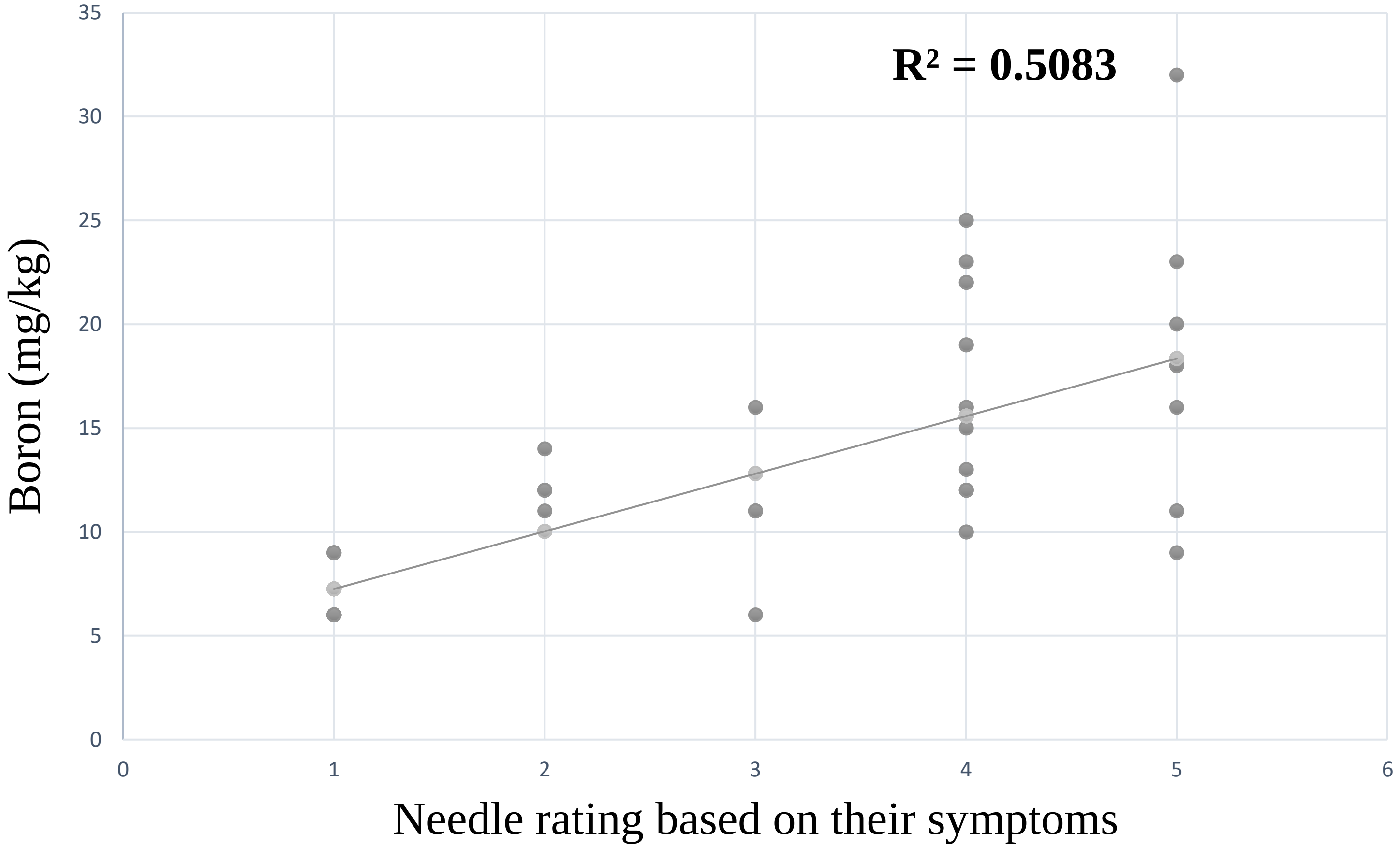
Relationships of Sulfur with BSNB severity

Results



Relationships of Sodium with BSNB severity

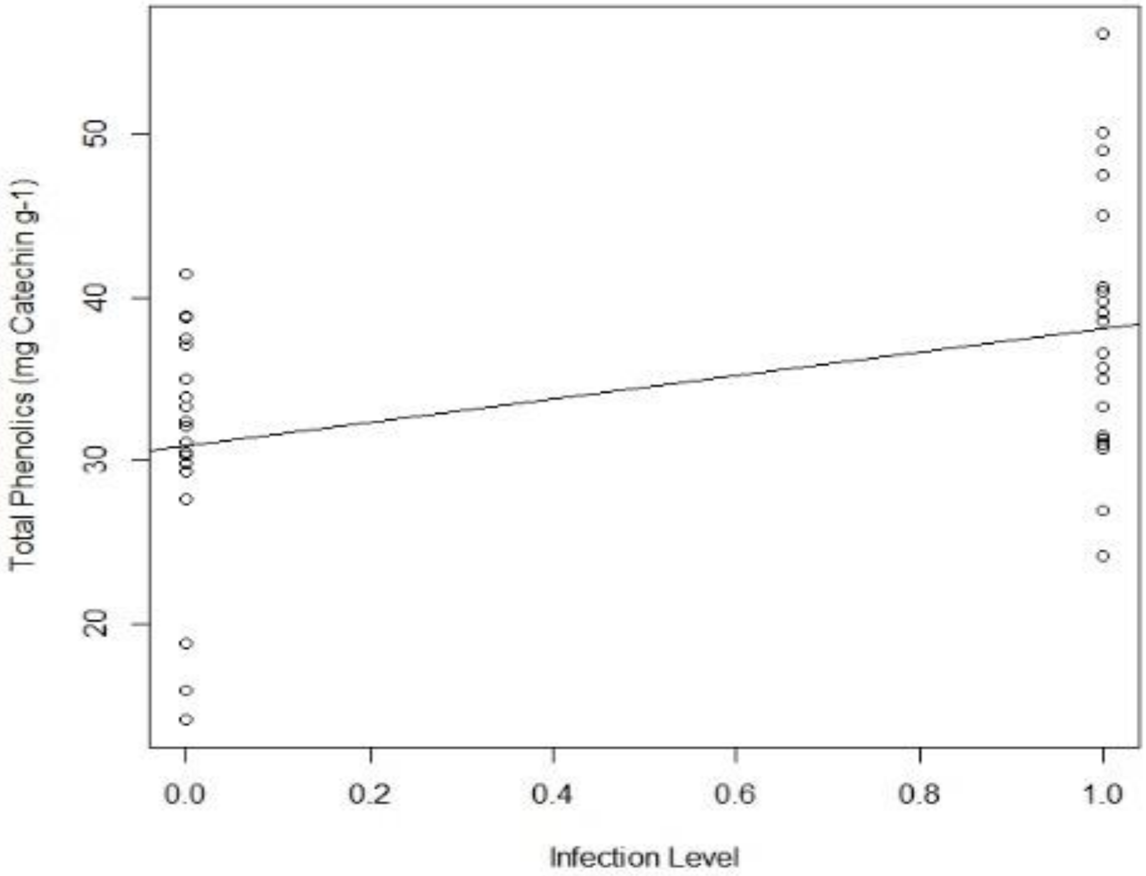
Results



Relationships of Boron with BSNB severity

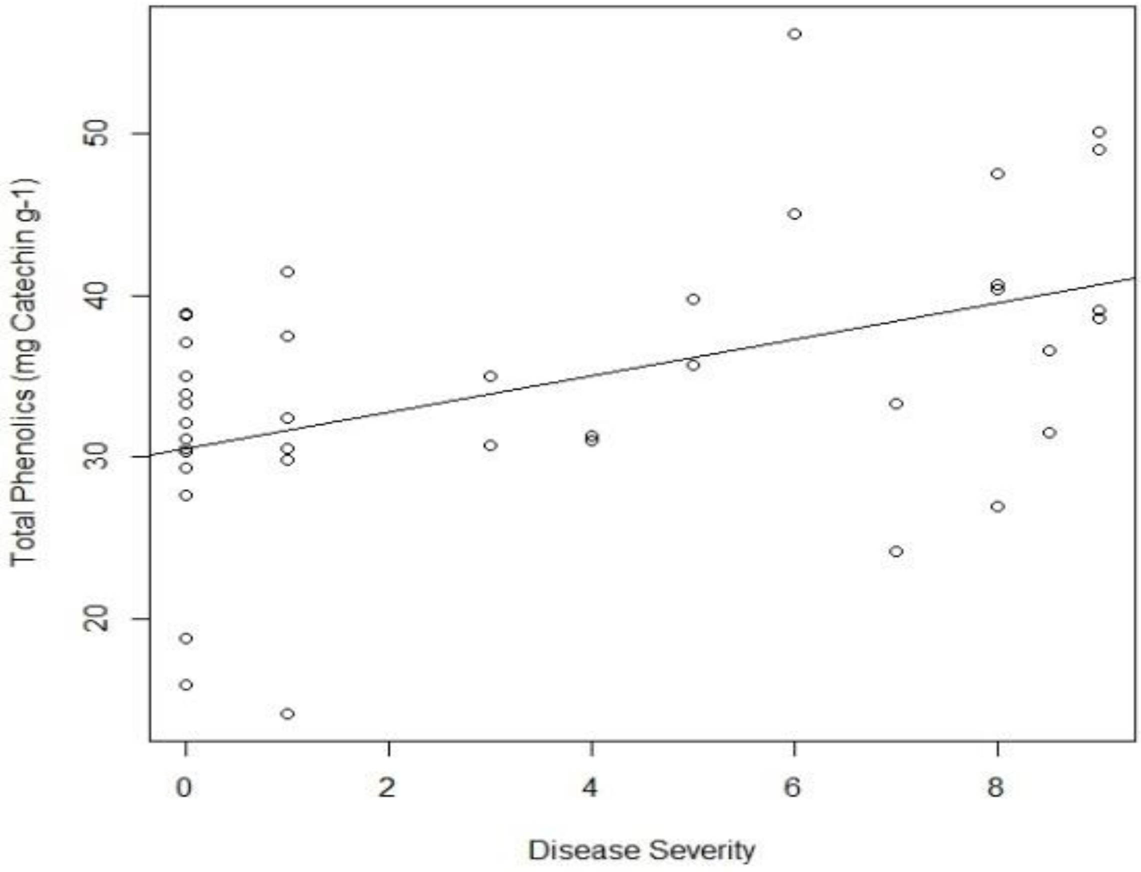
Results

A



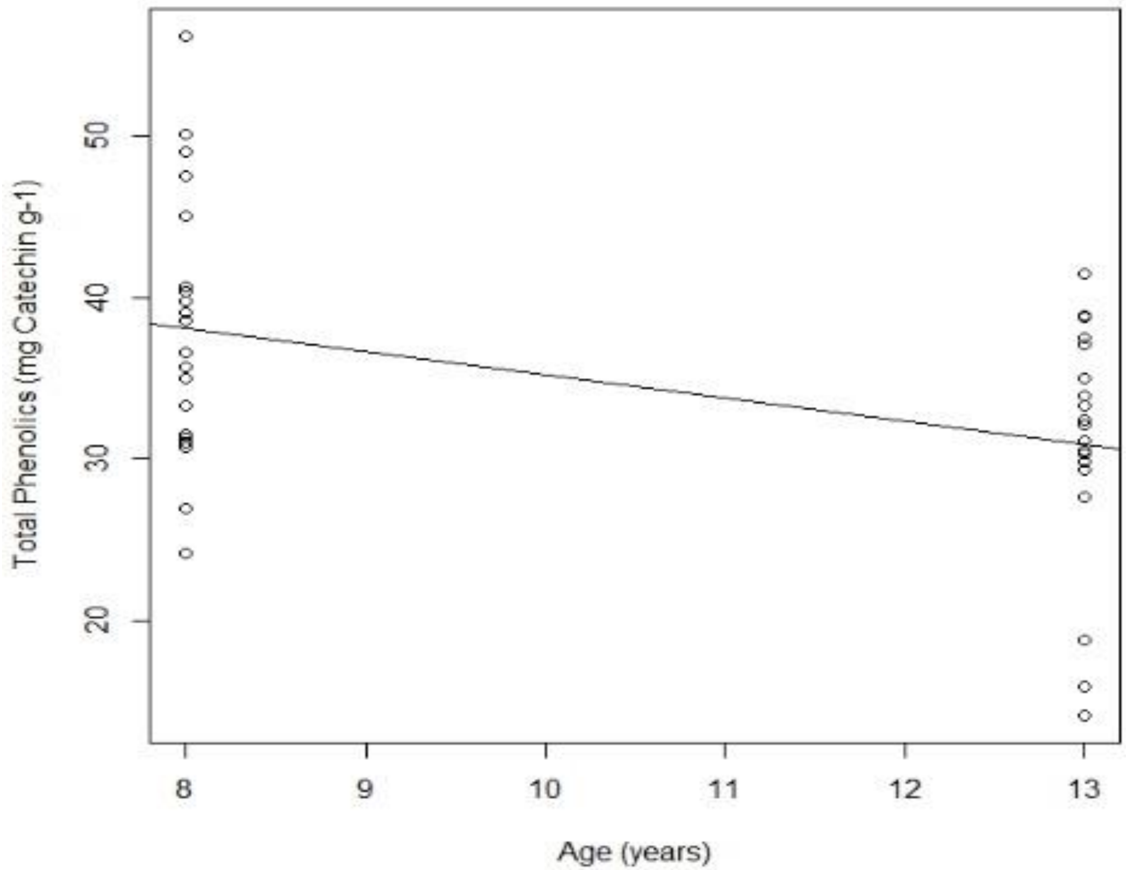
Relationships between total phenolics concentration and infection level

Results



Relationships between total phenolics concentration and disease severity

Results



Relationships between total phenolics concentration and age

Results

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
- pH is different

Conclusions

Lecanosticta acicola infection

- Loss of leaf area and carbon supply
- N and P is mobile
- Ca, B and Mn are immobile
- Loss of membrane integrity

Lecanosticta acicola impacts

- Loblolly pine foliage and foliar chemistry
- Total phenolics production as a normal defense system
- High concentration of nutrient contents

Future Directions of the Study

Management perspectives

- Development of inoculation protocol
- Resistant loblolly pine families
- Biomarker development
- Long-term monitoring plots

Research perspectives

- Population genetic study
- Whole genome sequence

Finally, BSNB must be monitored, forecasted, included in management strategies

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Mark Estrada
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Sylvester Menanyih
John Mensah
Jessica Ahl

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

Forest Health State Cooperative

WORKING TO KEEP TREES HEALTHY

Forest Health Dynamics Laboratory

College of Forestry, Wildlife and Environment – Auburn University