FOREST HEALTH COOPERATIVE MEETING - 2020

Isolation, Identification and Characterization of Fungi Associated with Loblolly Pine Needle Damage in the Southeastern US

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Background

Globally, increased tree mortality has been observed due to greater frequency and severity of the biotic attack and climatic stressors

Potential increases in tree mortality associated with climate-induced physiological stress and interactions with other climate-mediated processes such as disease and insect/pest outbreaks

Successive needle defoliation by pathogens and environmental stress can limit the tree's ability to take up water and nutrients, which can affect forest productivity and carbon stocks

Background

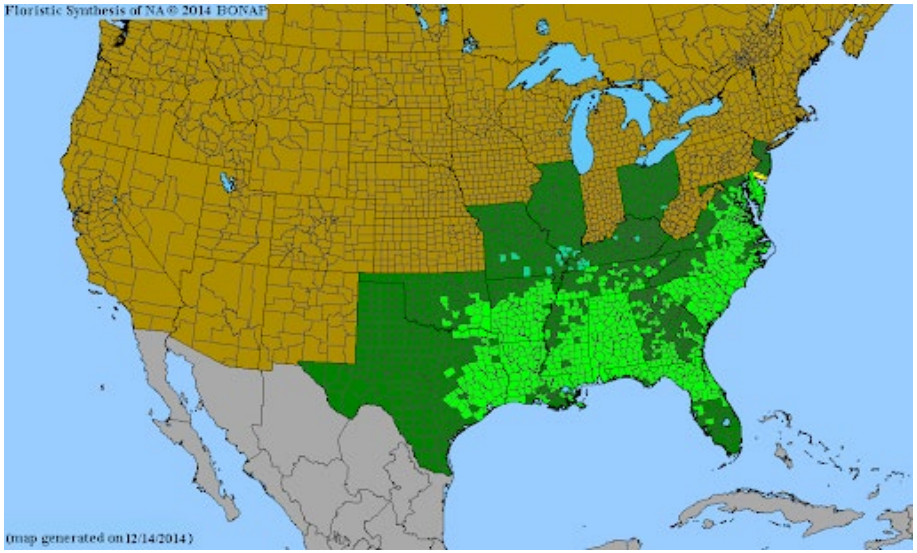
Loblolly Pine is a leading commercial timber species native to the southeastern US

Naturally, it grows in 15 southeastern states mostly in combination with shortleaf pine

An emerging problem of needle damage has been encountered over the past few years

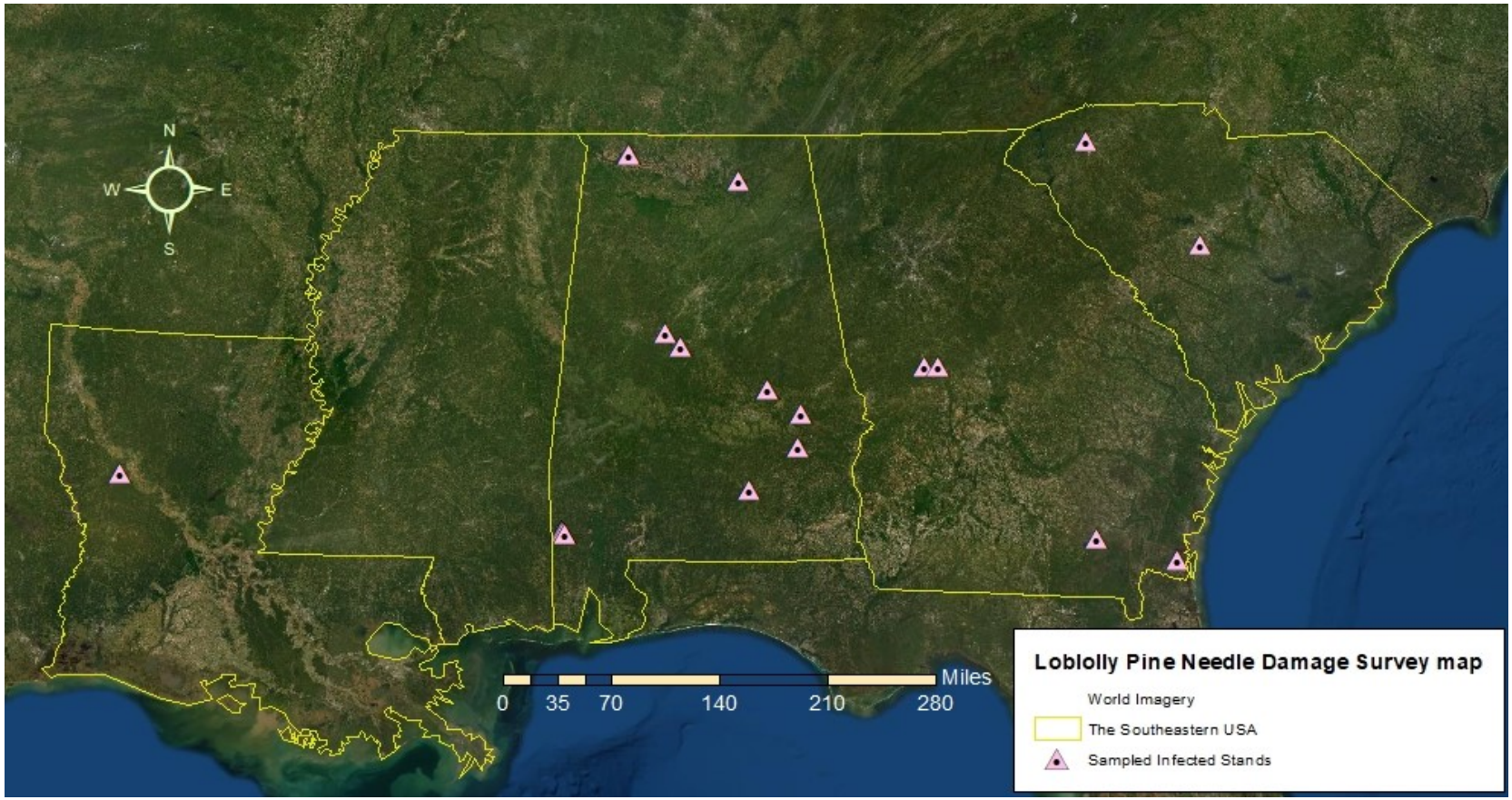
Symptoms typically range from needle discoloration, premature defoliation, thin crown followed by tree mortality

It is likely to be caused by single or a combination of several fungal pathogen (s)



Natural range of loblolly pine

Materials & Methods



Map of loblolly pine foliar damage observed in stands across the southeastern US

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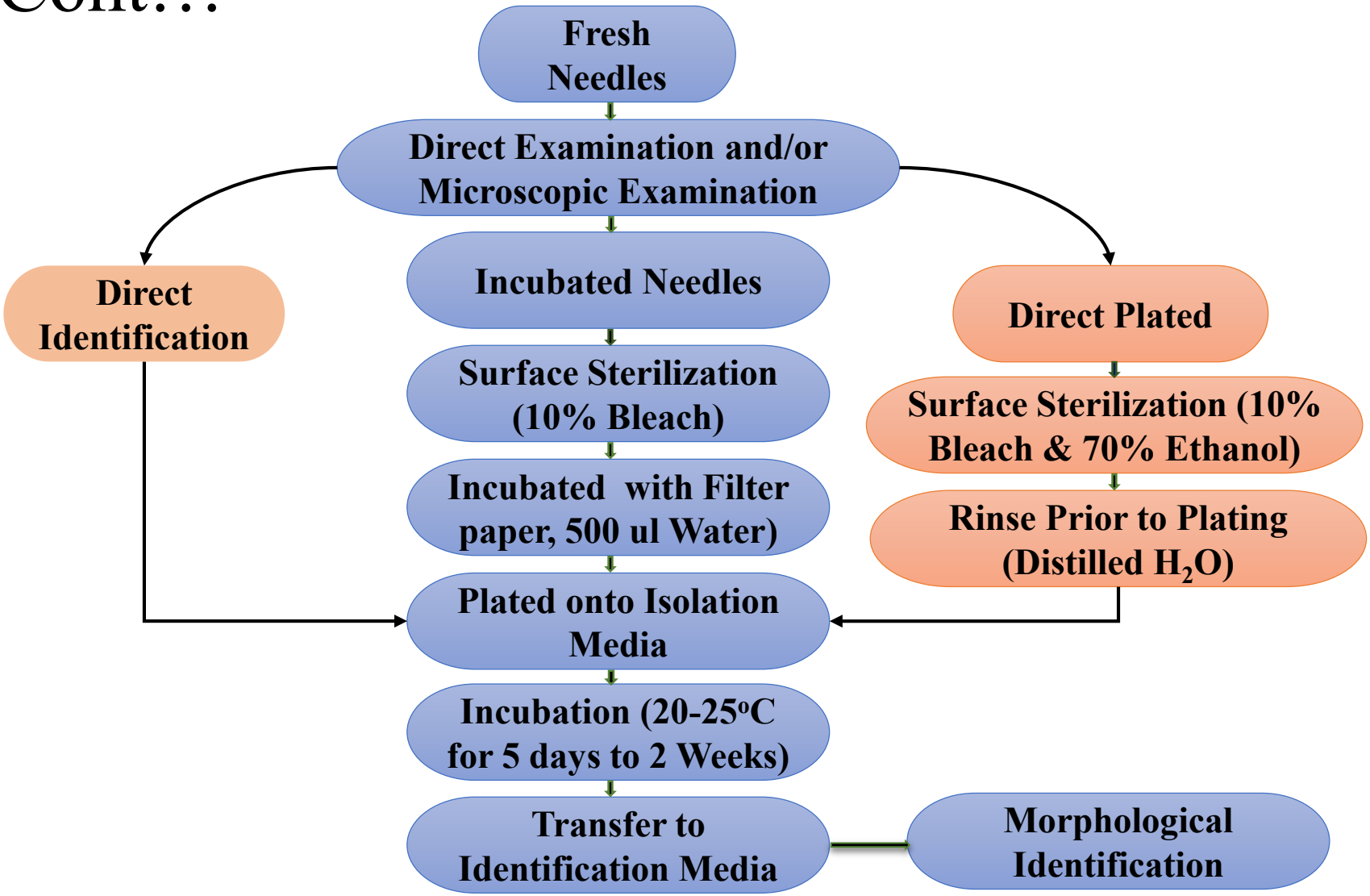

Cultural methods of fungi identification

Cont…

Growth media utilized for recovering fungi from needles

1. CMA-PARP (**Phytophthora** selective)
2. V8-Agar (**Phytophthora** selective)
3. Carrot Agar (**Phytophthora** selective)
4. DM+S, DSM+S (**Dothistroma** selective)
5. APDA & ¼ PDA (**Lophodermium** & other fungi)
6. Fresh PNA & Dried PNA (**Lecanosicta** selective & some other fungi)
7. 2% MEA (**Diplodia** selective & other fungi)

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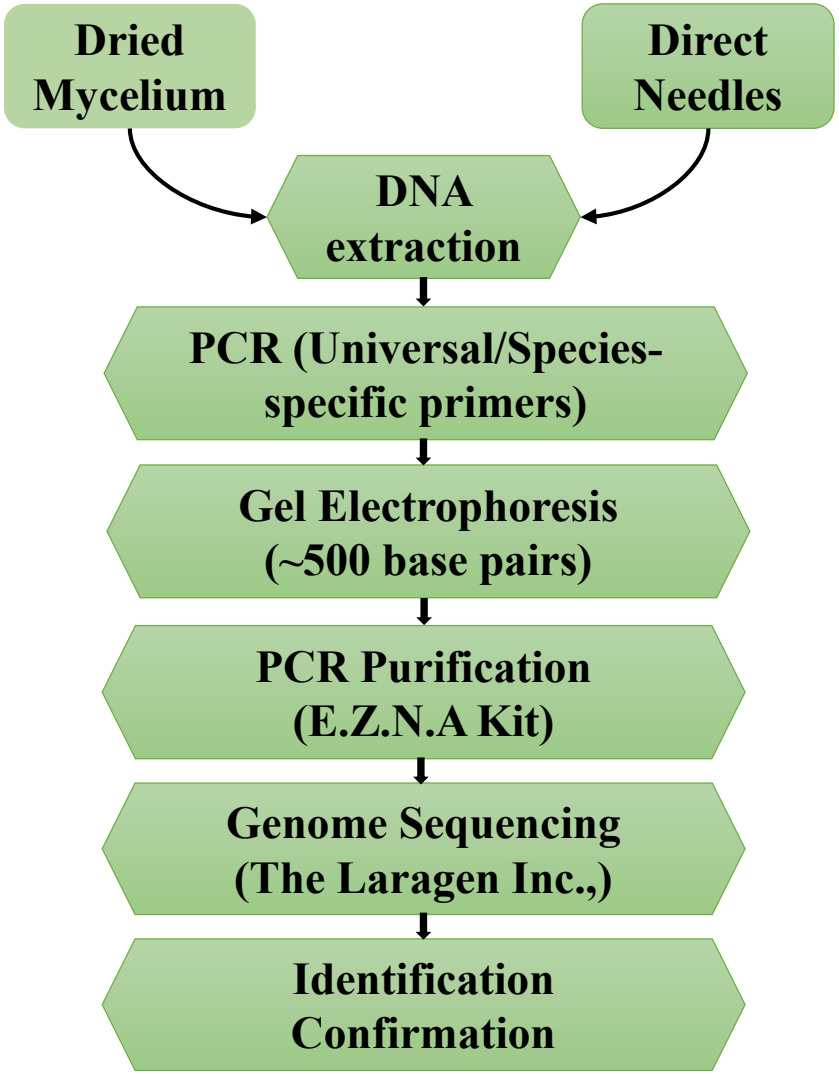

Molecular methods of fungi identification

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Phylogenetic analysis

Purified PCR products were sent to the Laragen Inc., Biotechnology for sequencing

Raw sequences were edited and adjusted using Bioedit software and deposited in GenBank and BankIT

Phylogenetic and molecular analyses were done by MEGA version 4.0 software

Alignments were completed using ClustalW

Trees were constructed using Neighbor Joining (NJ) and Maximum Likelihood (ML) method

1000 bootstrap replications were performed for NJ analysis

Loblolly Pine Health Monitoring

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

Less than a third of the crown affected (>1/3)
One-third to two-thirds of the crown affected (1/3 to 2/3)
More than two-thirds of the crown affected (<2/3)

The location of the crown damage was also recorded:

Bottom, middle, or top of the crown

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

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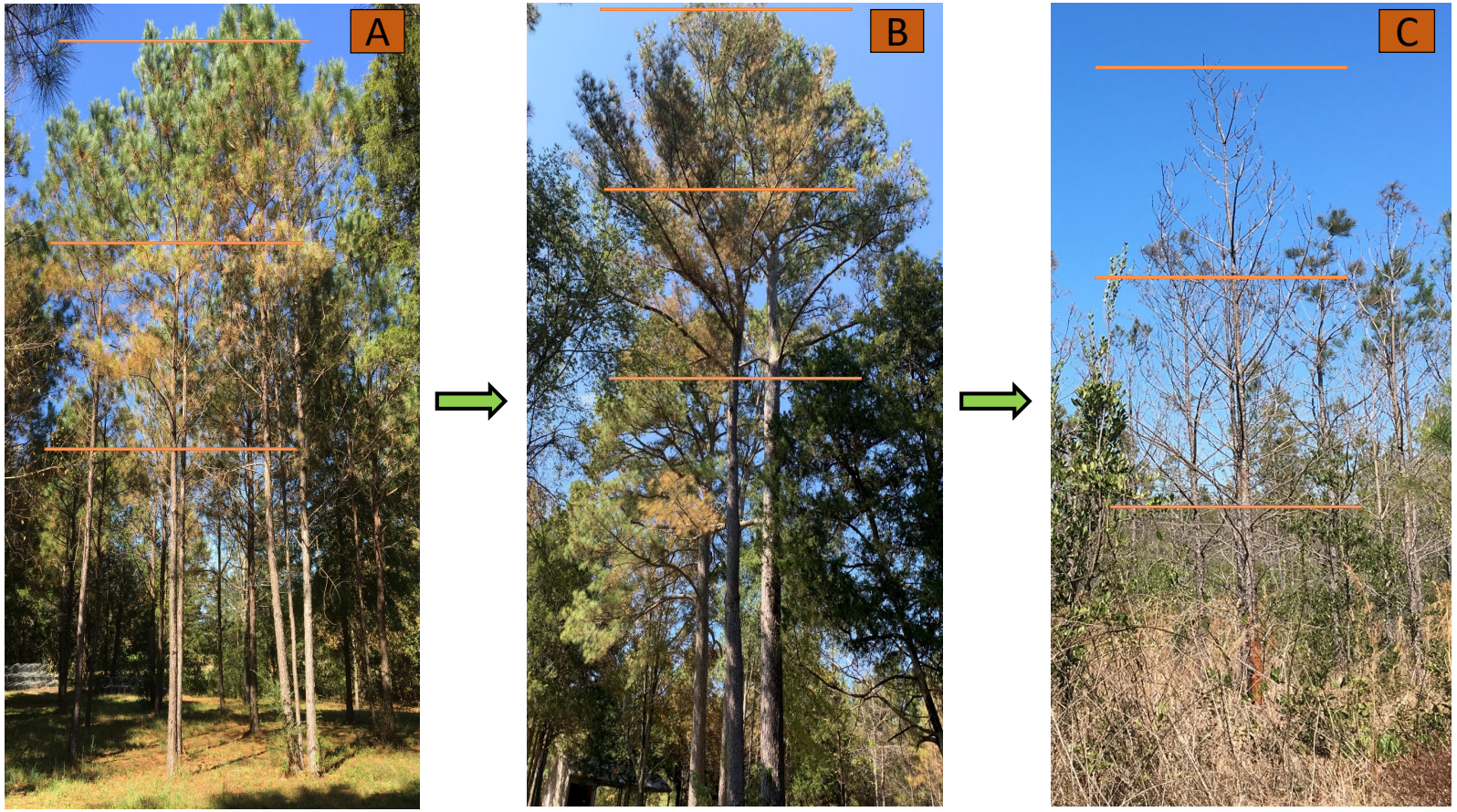
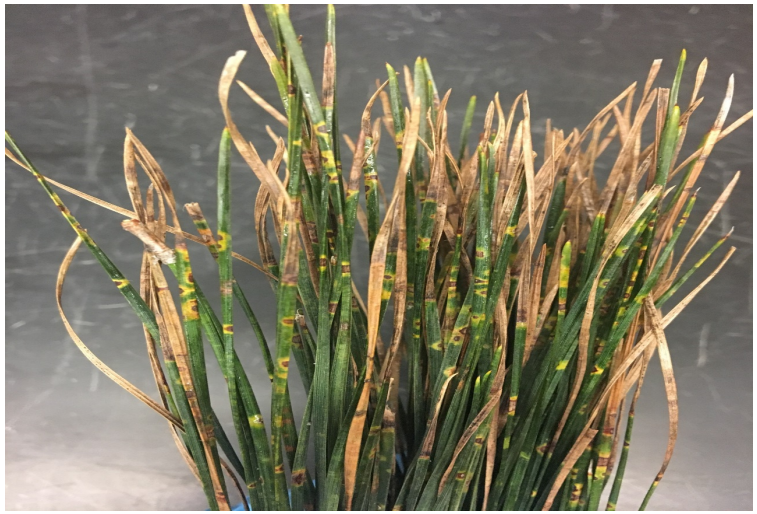


Crown severity rating such as **(A)** One-third of crown infected **(B)** One-third to two-third of crown defoliated/infected & **(C)** More than two-third to full crown defoliated/infected

Results



Region of occurrence

Washington, Colbert, Elmore, Madison, Crenshaw (AL)
(12 infected stands)

Symptoms

Yellow to brown spots, premature defoliation, thin crown

Pathogen (s) recovered

Brown-spot needle blight, *Lecanosticta acicola*
Rhizosphora needle cast, *Rhizosphora kalkhoffii*
Neopestalotiopsis clavispora
Epicoccum nigrum

Endophyte

Cont…

Region of occurrence

West Crenshaw County, (AL)

Symptoms – Yellow banding, defoliation & dying trees

Pathogen - Brown-spot fungi ???

RMS Mystery "disease", West Crenshaw Cty

Acres impacted = 348

				Treatment/Yr				
Cmt	Stand	Acres	Age	Site Prep	Est Year	HWC	DAP/Urea FT	DAP/Urea FT
1365	3	84	15	2004	2005	2005	Spring 2019	
1365	7	57	13	2006	2007	2007		
1365	9	104	13	2006	2007	2007	Spring 2019	
1369	9	103	16	2003	2004	Tank Mix HWC	Spring 2011	Spring 2019
		348						

Cont…

Region of occurrence

Upson (GA)
Hampton (SC)
(3 infected stands)

Symptoms

Brown needles at the tip, death of the terminal buds, needle defoliation

Pathogen (s) recovered

Diplodia tip blight, *Diplodia sapinea*
Diplodia seriata

Endophyte

Trichoderma caerulescens
Hormonema macrosporum

Cont…

Region of occurrence

Bibb (AL)
Ware & Camden (GA)
(3 infected stands)

Symptoms

Dead and dying needles, football shaped fruiting bodies, needle defoliation

Pathogen (s) recovered

Lophodermium needle cast, *Lophodermium* spp.

Sydowia polyspora
Hendersonia pinicola
Endophyte

Cont…

Region of occurrence

Macon & Colbert (AL)
Nachitoches (LA)
(4 infected stands)

Symptoms

White aecia, browning needles, defoliation

Pathogen (s) recovered

Pine needle rust, *Coleosporium spp.*
Brown-spot needle blight, *L. acicola*

Epicoccum nigrum
Fungal endophyte

Endophyte

Cont…

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

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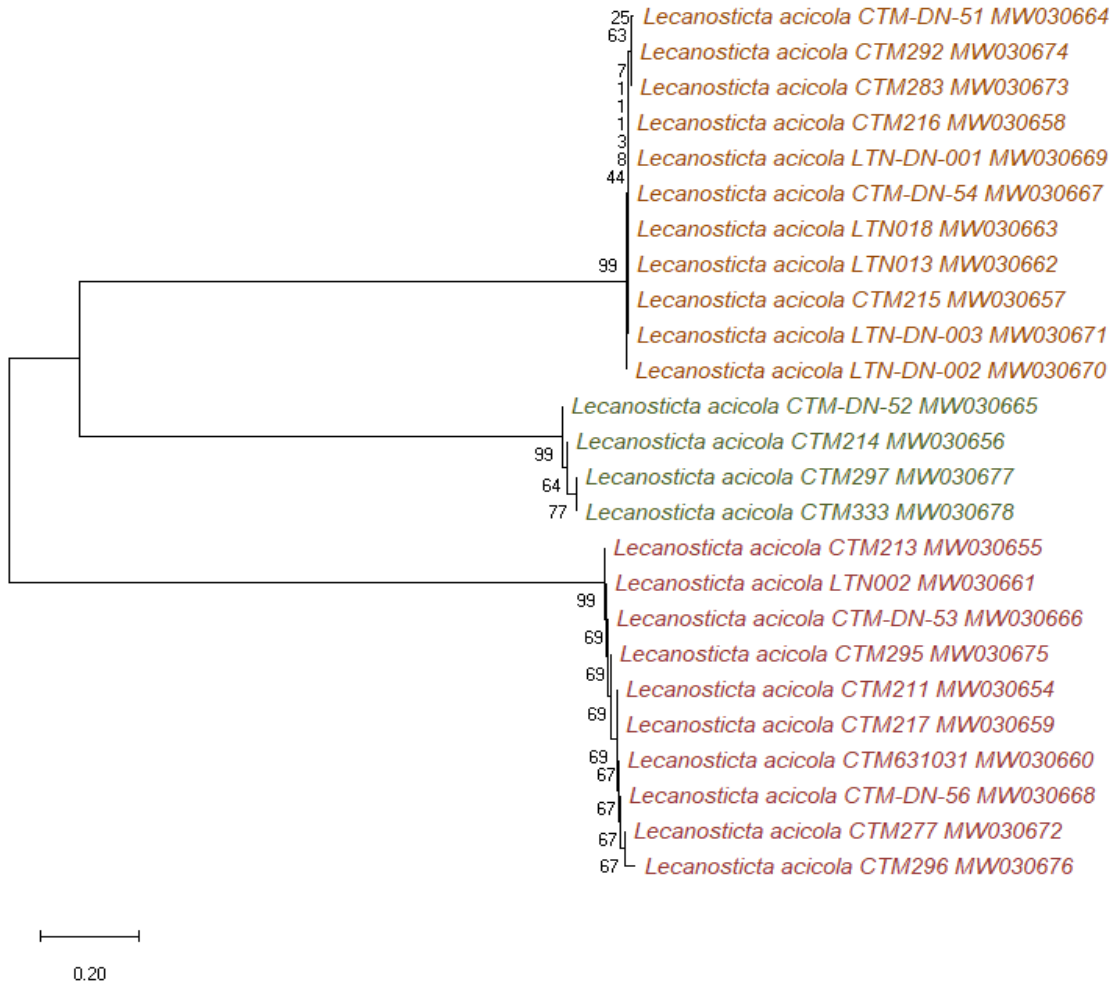

Maximum likelihood (ML) tree representing *L. acicola* and three lineages of *L. acicola*

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Total 701 fungal cultures were recovered from unhealthy loblolly pine needles

They are divided into 58 major groups based on their colony morphology

To date, 28 species of fungi representing 17 families were identified followed by morphological and molecular data

Among them, 12 species of fungi represent either pathogens or weak parasites

Conversely, 8 species of fungi represent endophytes, and 8 species represents saprophytes

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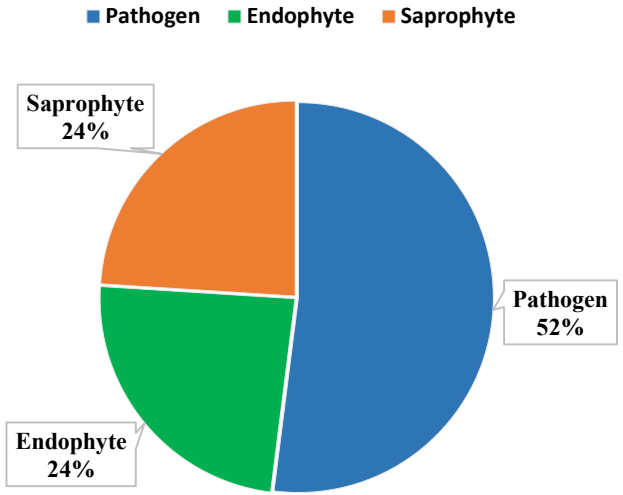

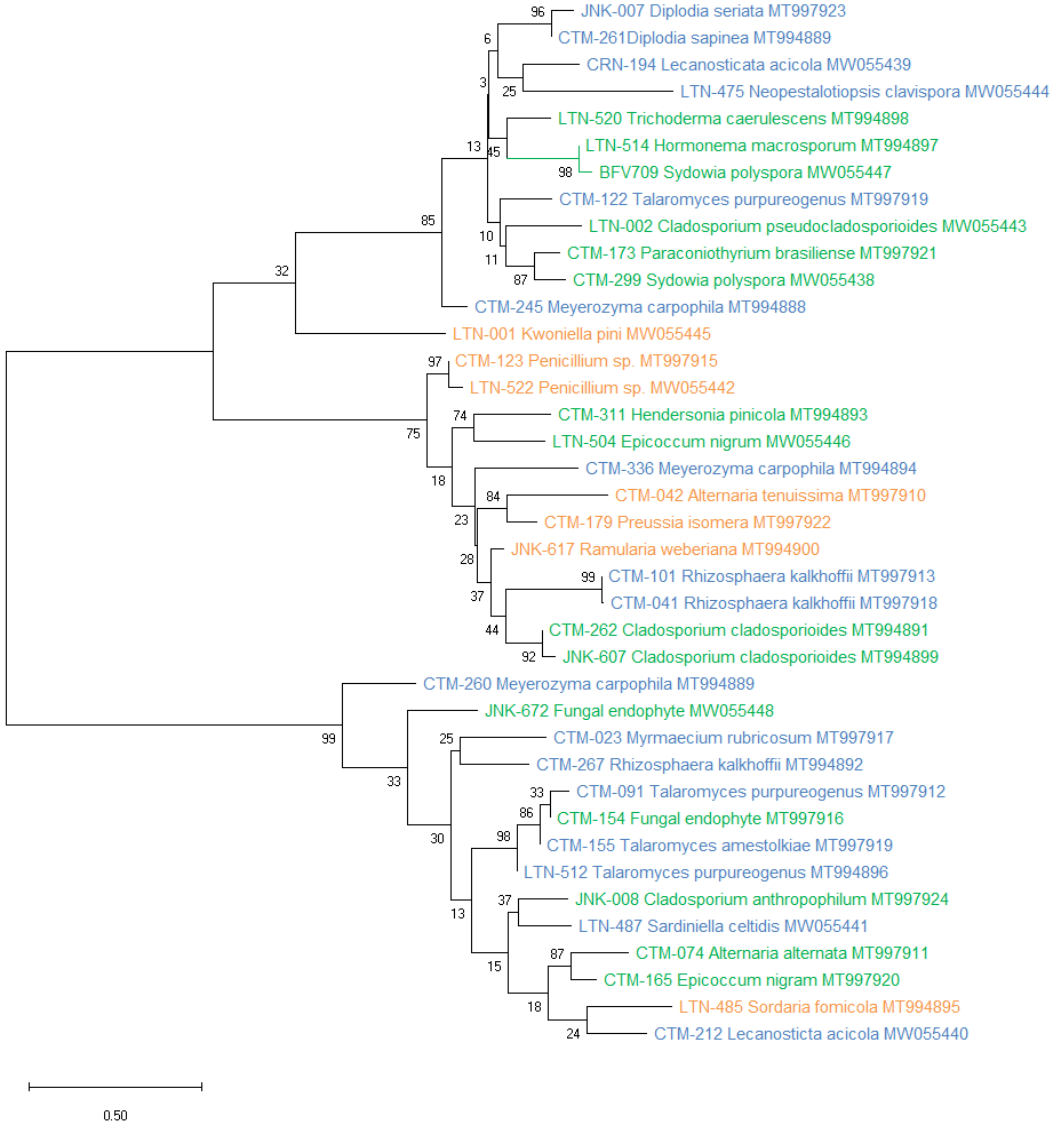

Neighbor-joining tree representing pathogenic, endophytic & saprophytic fungi found in the needles

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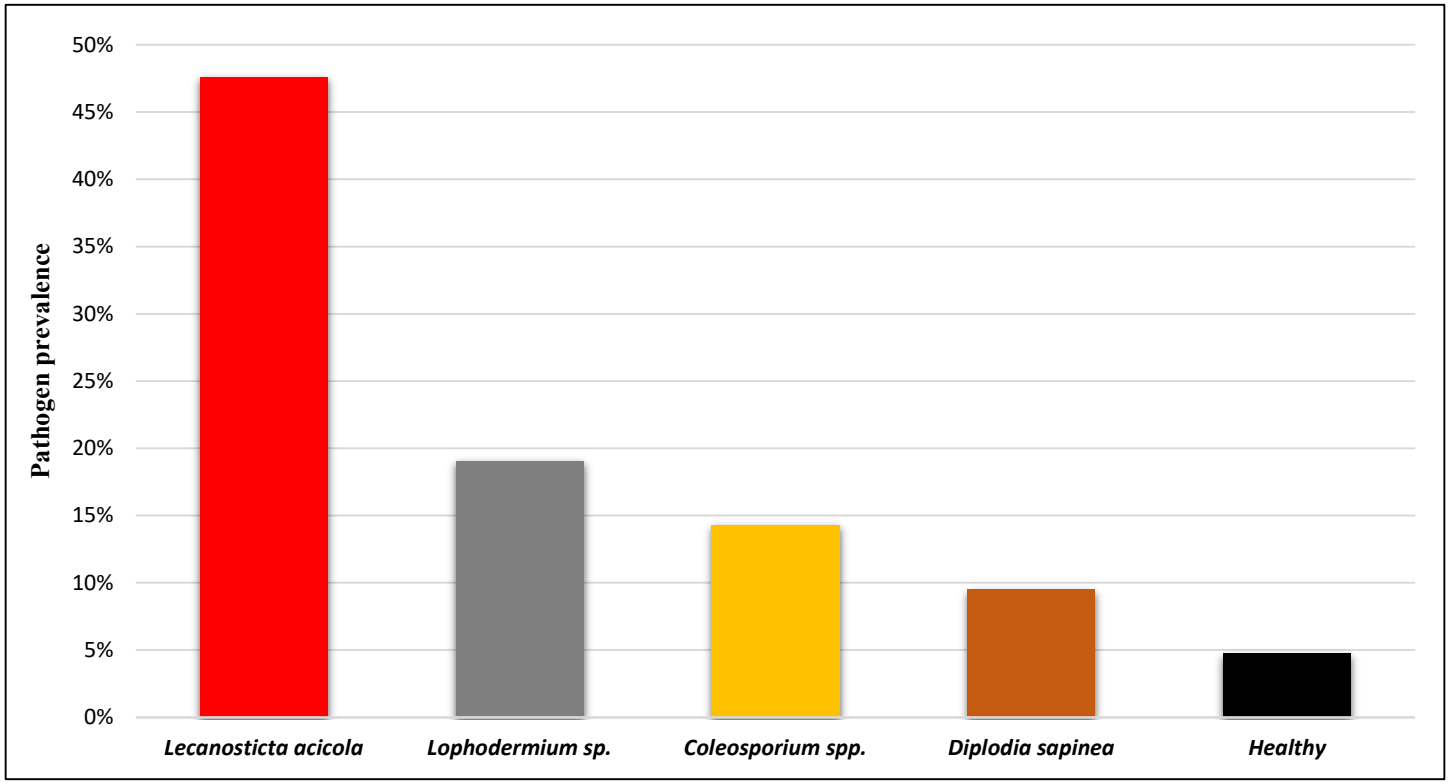

Pathogen prevalence of *L. acicola*, *Lophodermium* sp., *Coleosporium* sp., *D. sapinae,* and healthy sites

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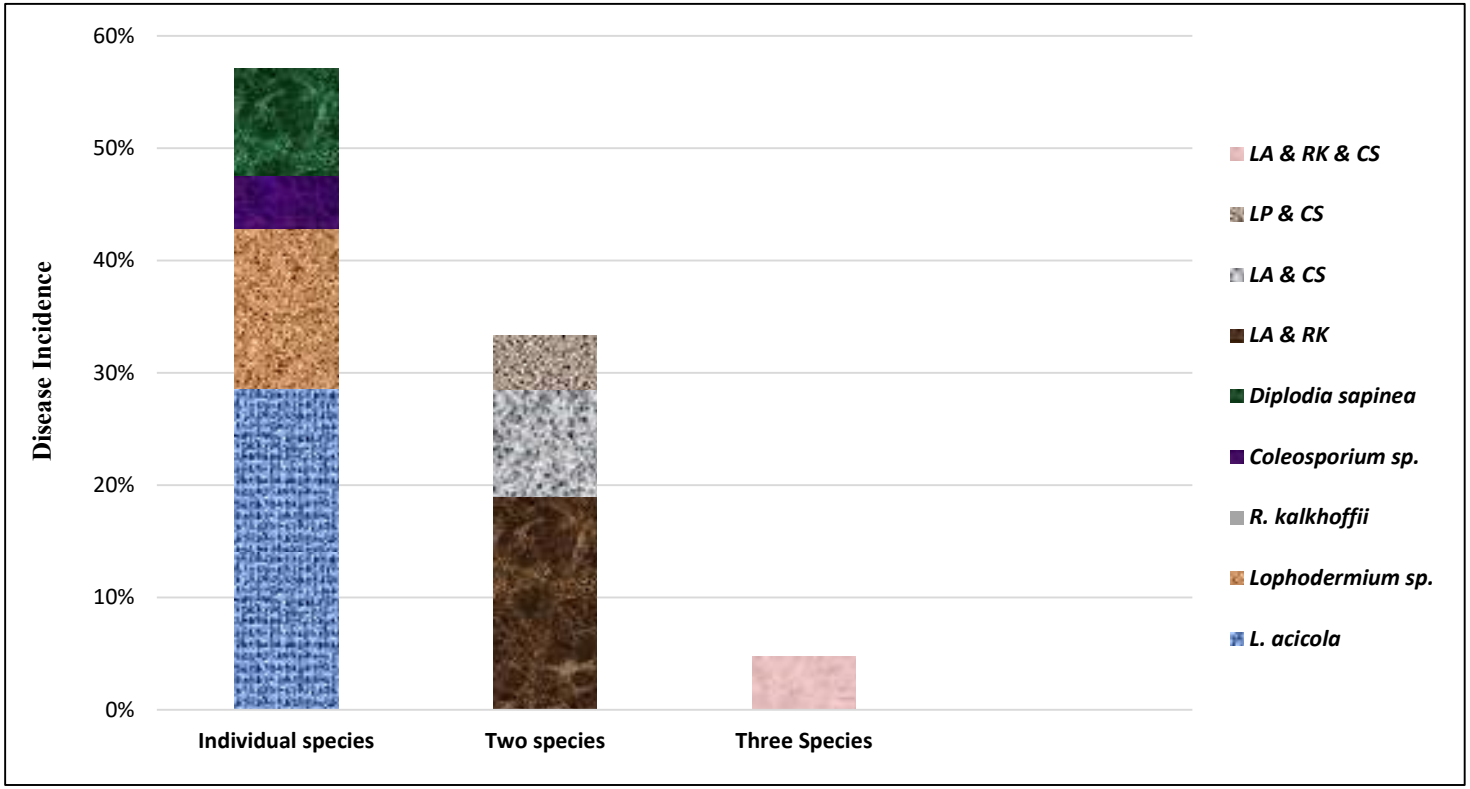

Pathogen prevalence of each species and all species combinations

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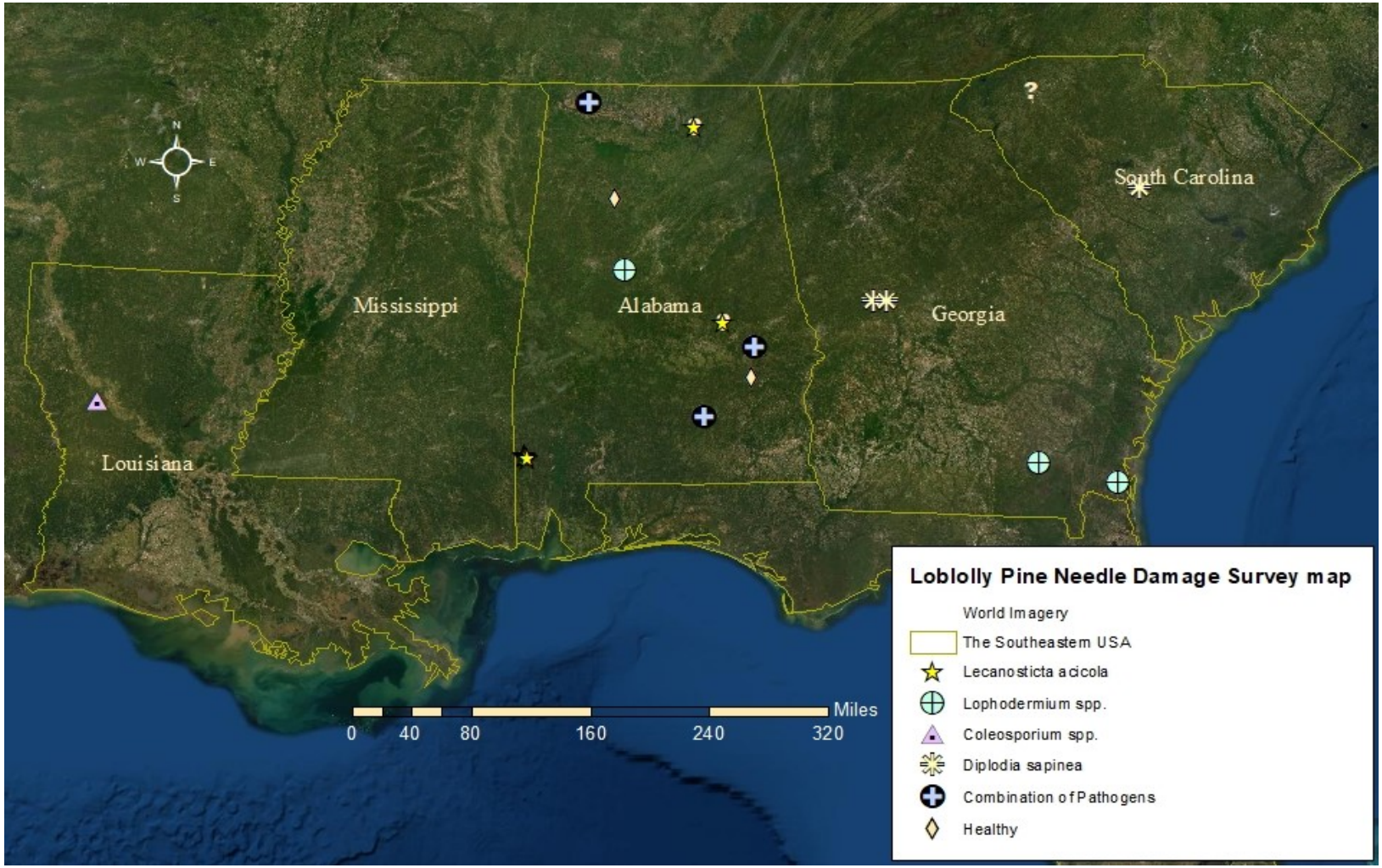
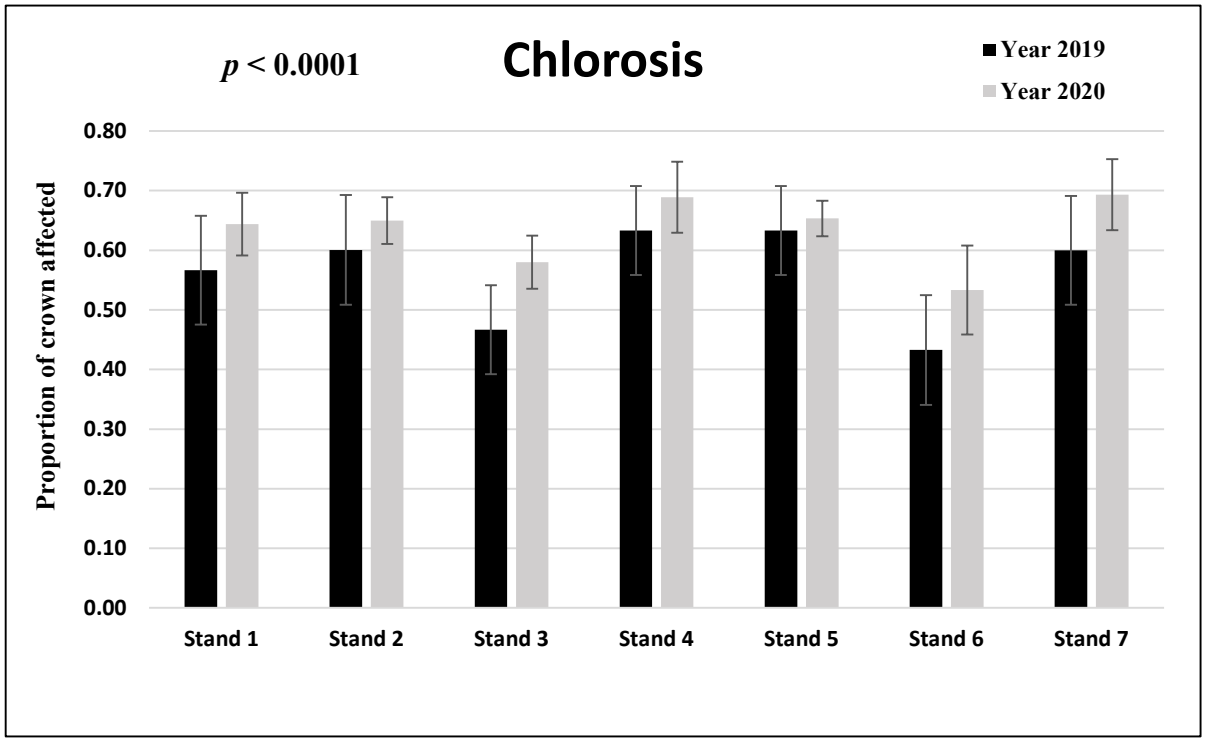


Map of loblolly pine foliar damage observed in stands across the southeastern US

Loblolly Pine Health Monitoring



Frequency of chlorosis of healthy and unhealthy loblolly pine at Chatom, AL at summer 2019 and 2020. Bars represent means and standard errors (N = 70 trees)

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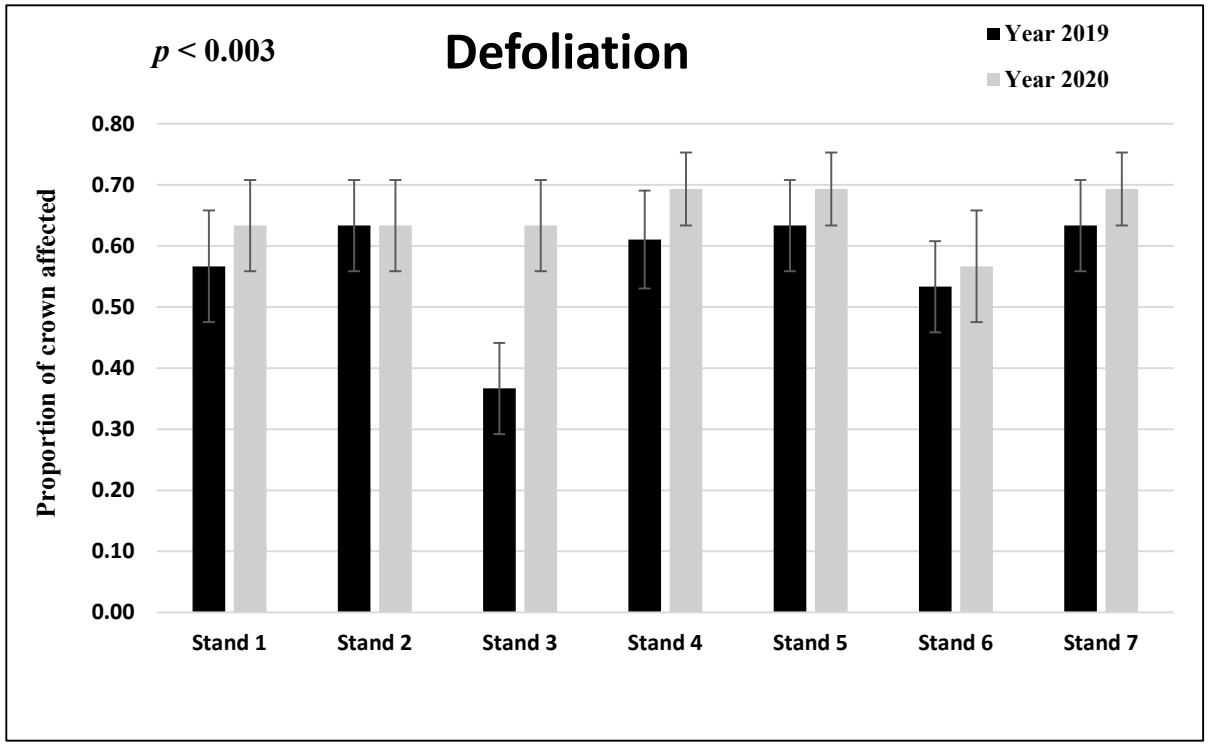


Frequency of defoliation of healthy and unhealthy loblolly pine at Chatom, AL at summer 2019 and 2020. Bars represent means and standard errors (N = 70 trees)

Discussion

LPND in the southeastern US is due in larger part to the brown-spot pathogen *L. acicola*, in addition to needle cast *Lophodermium* spp., needle rust *Coleosporium* sp. and tip blight *D. sapinae*

It also can be caused by a combination of pathogens such as *L. acicola* and *Coleosporium* sp., *L. acicola* and *Lophodermium* sp., *L. acicola* and *R. kalkhoffii* as well as *L. acicola*, *Coleosporium* sp., and *R. kalkhoffii*

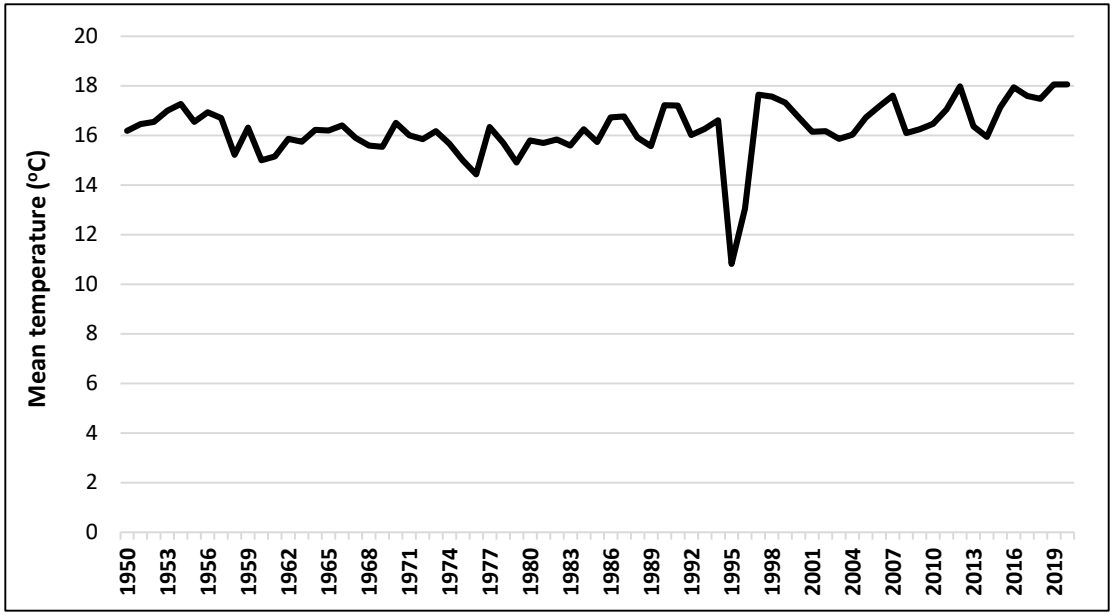
Pathogen (s) are localized in terms of the needle damage respective to the loblolly pine

Affected trees are repeatedly symptomatic (chlorotic, defoliated) every year

The disease is spreading from symptomatic trees to initially healthy trees

Discussion

Stands infected by the brown-spot pathogen are mostly situated in the moist areas. Stand prevalence on those sites ranged from 55% to 99%. Site conditions could explain why stands are experiencing worse conditions on these sites.



Mean temperature around seven long-term monitoring plots in Chatom, Washington, AL from 1950-2019

Further Activities

The study is still investigating the pattern of the disease and nature of spreading across states, locality, and/or region

The study is expected to expand field-based sampling across the southeastern US as well as get more samples from landowners, forest managers, and forest health state cooperators

The study is aiming to construct a more detailed distribution map of needle pathogens across the southeastern US

The research continues to associate the environmental factors which may drive the emergence and spread of LPND

The study is under way analyzing habitat suitability of needle pathogens across the southeastern US

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REGIONS


KEEPING TREES HEALTHY