Title: Identifying the fungal pathogens associated with loblolly pine needle damage in the southeastern USA.

Background

The loblolly pine needle defoliation (LPND), suspected to be caused by multiple fungal pathogens, is threatening the ecological health of the southeastern forests and economic vitality of the region's timber industry. It causes stunted tree growth, reduced photosynthetic potential and eventual mortality of the infected trees. It was first surveyed in Chatom, Washington, Alabama in spring 2019. Currently, it is affecting more than 1000 hectares of loblolly plantations under Stallworth Land Company (Stallworth, personal communication). Adjacent stands are at high risk due to the ongoing spread of this disease. Both mature and regenerated trees are infected by the disease. Therefore, it has become a concern to some plantation owners in the southeast USA. The study seeks to assess the incidence, severity and or the causal fungal pathogens associated with LPND.

Figure 1. Affected *Pinus taeda* A) severe defoliation; (B) dead tree fallen on the ground; (C) thin crowns; and (D) mature and regeneration are both affected.

Approach

Loblolly pine (*Pinus taeda*) needle samples were collected from the infected stands and brought into the Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, Auburn University from March to October 2019 and February to March 2020. All branch tips were visually examined for fungal fruiting structures. Disease incidence and severity were recorded. Twenty needles from one representative branch tip per tree were placed in a moist chamber, incubated at 25˚C for 24 to 72 hours, and then examined with the aid of dissecting and light microscopes. Petri plates consisted of incubated needles with filter papers moistened with 500µl deionized water and sealed with Parafilm. Diseased needles were surface sterilized with a 10% bleach solution for 1 min, rinsed three times with de-ionized water and allowed to dry in a fume hood. Dry needles were then plated onto various kinds of media such as cornmeal agar amended with Pimaricin, Ampicillin, Rifamycin and PCNB (CMA-PARP), Dothistroma Sporulating Media (DSM) amended with streptomycin, 2% Malt Extract Agar (MEA) and incubated at ambient temperature.

Results

A total of seven stands were sampled in Chatom, Alabama. All of them were plantation stands and located in moist areas. Soils are mostly sandy clay to clay in these sites. Samples were taken from 172 trees of all age classes. To date, a total of 22 fungal strains were isolated, and fungal isolates exhibiting analogous phenotypic and morphological markers were grouped, thus arriving at 7 isolates for molecular analysis. Sign and symptoms of brown spot needle blight, BSNB (caused by *Lecanosticta acicola*) were frequently observed in the infected needles (Fig. 2).

Figure 2. Brown spot needle blight fungi, *Lecanosticta acicola*, causes (A) chlorosis with resin-soaked spots (B) necrotic bands limited to less than one-third of the needles (C) premature defoliation of the infected needles from the *Pinus taeda* trees.

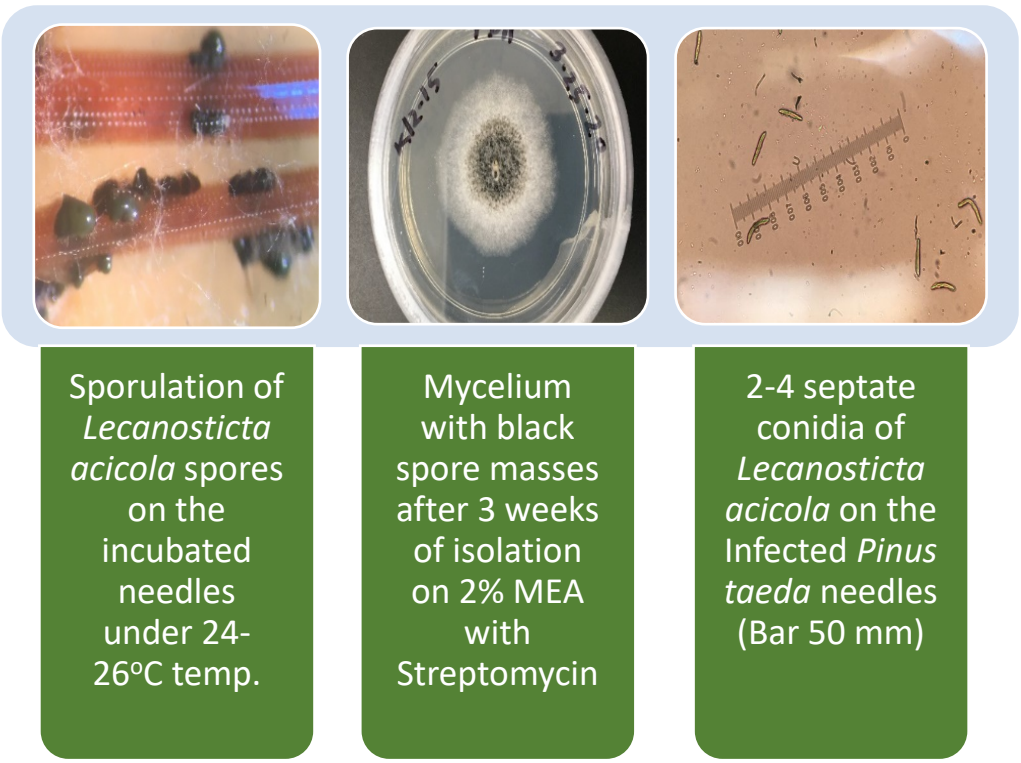

Figure 3. Pathogenic brown-spot needle blight fungi, *Lecanosticta acicola*, growth on incubated infected needles and on 2% MEA media. Black fruiting bodies and conidial masses are observed with the aid of light and dissecting microscope.

However, several other opportunistic fungi were found on the infected needles, but these were not directly associated with needle blight symptoms and considered as secondary invaders. As per study concern, tree death mightn't be direct consequence of BSNB rather promoted probably by these opportunistic organisms after years of defoliation. Among them, *Pestalotiopsis* was the most frequently recovered fungi present in the infected needles. *Alternaria*, *Trichoderma, Penicillium*, *Aureobasidium*, *Epicoccum* spp. were also observed (Fig. 4). Disease severity is higher in the younger stands and ranging from 30% to 65% of the infected trees. The 2012 stands are experiencing more disease consequences than other older stands.

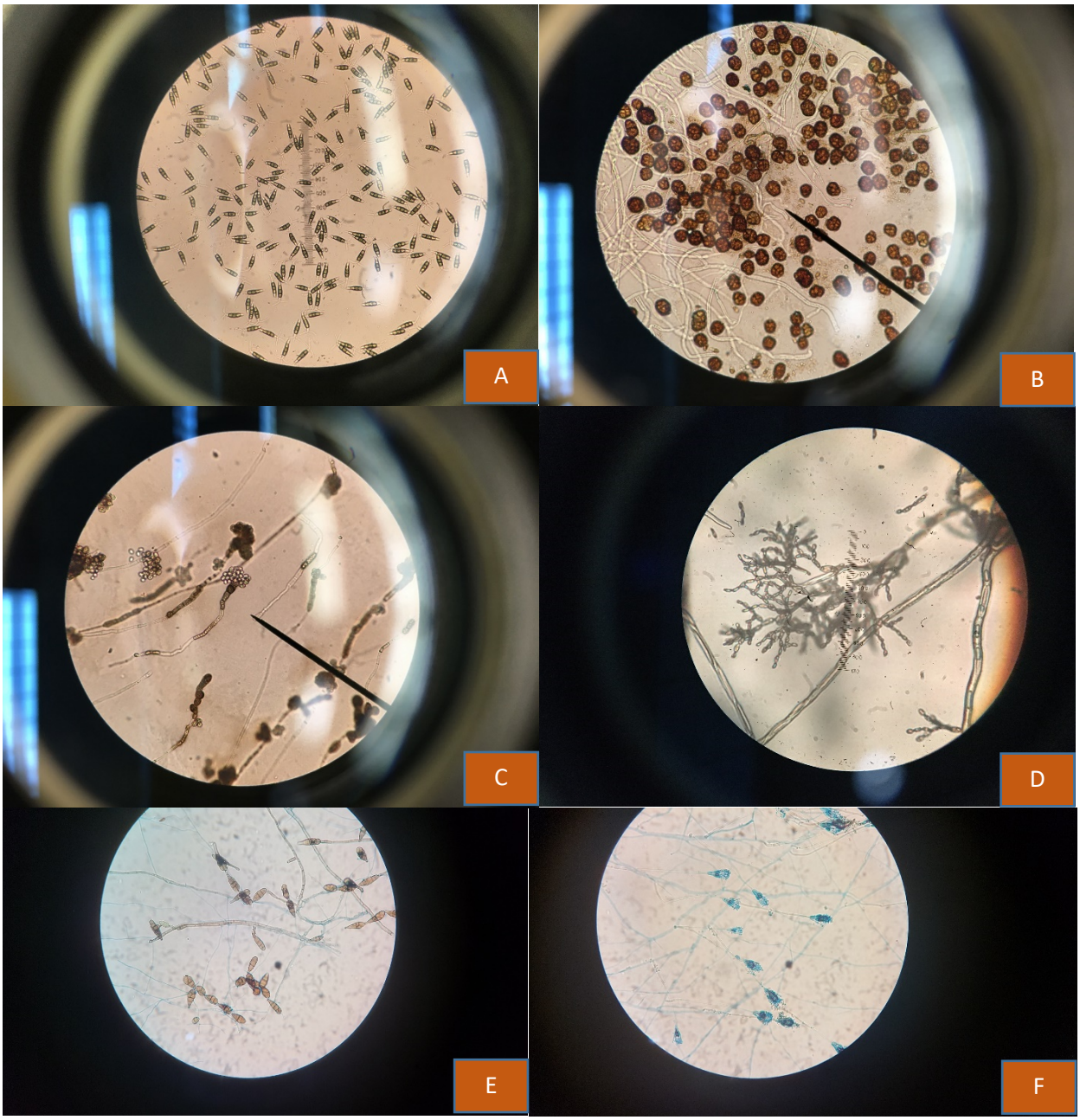

Figure 4. Opportunistic fungi (A) *Pestalotiopsis* spp. (B), *Epicoccum* spp. (C), *Aureobasidium* spp. (D), *Trichoderma* spp. (E), *Alternaria* spp. & (F) *Penicillium* spp. found in the infected loblolly pine needles.

Current Activity

The study requires more sample analysis throughout the southeast to understand brown spot threat and expansion range to the southeast USA. Therefore, the frequency of collecting data from the infected stands is increased to examine the BSNB impacts on the southeast. Moreover, three more media have been added in the methods such as acid potato dextrose agar (APDA), pine needle media (PNM), water agar for determining the causal pathogens. Currently recovered fungi are carefully chosen for molecular analysis to confirm our results. Apart from this, fusiform rust is currently found largely spreading into all the infected stands and developing enormous galls onto the infected trees. It is regarded as one of the most serious disease of pines in the southern United States. Fusiform rust infection resulted in swellings on the infected branches and stems called galls. Galls vary in size and appearance but in uniform shape called fusiform. This spring, yellow bright aecial pustules were also found developing in the surface of the galls (Fig. 5). Below are the pictures of fusiform rust symptoms in the infected trees.

Figure 5. Fusiform rust causes (A), (B), (C) spindle-shaped gall on the branches of the infected loblolly pine trees & (D) bright yellow aecial pustules of the pathogen on the surface of the branch.