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Lecanosticta acicola isolation protocol

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ABSTRACT

A guide to the reproduceable isolation of *Lecanosticta acicola,* the causal agent of brown spot needle blight of pines.

Lecanosticta acicola is a well-known aggressive foliar pathogen that attacks pines, causing brown spot needle blight. Although it is a native pathogen of *Pinus palustris* in the southeast United States and an important pathogen of *Pinus sylvestris* Christmas tree farms, its behavior on many other *Pinus* species is poorly understood. *Lecanosticta acicola* is increasingly reported in associated with outbreak-scale damage on native pines, including *Pinus taeda* and *Pinus strobus*, throughout southern and eastern North America. The fungus is notoriously difficult to isolate in culture due to its slow growth and poor ability to compete with saprophytic microorganisms on nutrient-rich culture media. This protocol describes a reproducible method to obtain *L. acicola* isolates from *Pinus taeda* foliage for further study.

Needles were obtained from trees that displayed visible BSNB symptoms, including necrotic spots and bands bordered by yellow chlorotic zones and dead needle tips (Figure 1). Needles were incubated in a moist chamber (Ziploc bag with a damp paper towel) for at least 24 hours. Samples that were not overgrown with saprophytic fungi were allowed to incubate for several weeks. Chlorotic and necrotic spots and bands of individual needles were inspected for evidence of fungal fruiting, black acervuli erupting from the surface of the needle (EPPO Standards, 2015).

Figure 1. *Pinus taeda* foliage displaying brown spot needle blight symptoms, including the namesake necrotic spots.

Fruiting bodies were excised from the needle with a sterile scalpel and transferred to a glass microscope slide. Slides were prepared by wiping with 70% EtOH, drying, and then placing one drop of sterile DI water in the center. The fruiting body was placed in the sterile water and crushed gently with a coverslip. The slide was then examined under a compound microscope (Figure 2). Acervuli and conidia morphologies were compared to references (EPPO Standards, 2015; CABI; van der Nest *et*

al., 2019). In most cases, acervuli were observed emerging from within or just outside old bands and always from necrotic tissue. Microscope slides that contained fruiting structures similar to *L. acicola* were retained for isolation.

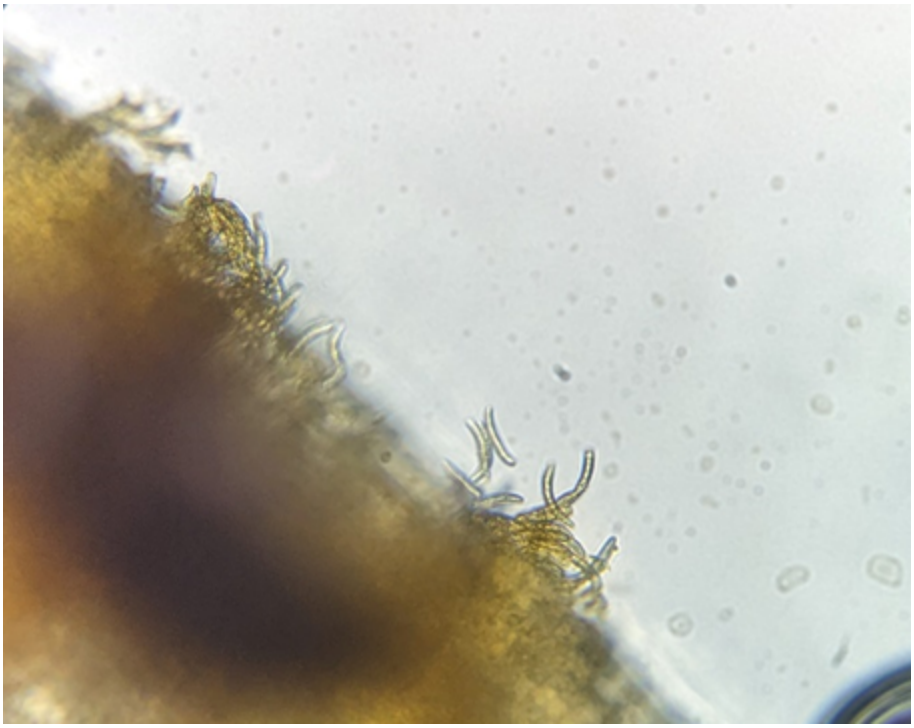

Figure 2. *Lecanosticta acicola* conidia emerging from an acervulus that formed within *Pinus taeda* foliage, viewed at 400 x magnification.

The coverslip was then carefully removed, leaving the water on the glass slide. A sterile inoculating loop was used to capture the fruiting body and a small amount of water. This suspension was then streaked for isolation (as with yeast or bacteria) on 2% malt extract agar (MEA) (Modified from Mullett and Barnes, 2012). A second loopful was streaked on ground pine needle agar (gPNA, modified from Luchi *et al.,* 2007) prepared with 2% w/v agar and 8% w/v ground *Pinus palustris* needles. Plates were wrapped in parafilm and incubated on an open shelf under ambient conditions. Plates were inspected for growth once daily. Some single spore *Lecanosticta* colonies became visible after two days, and all were apparent within seven days after inoculation. Nascent colonies displayed hyaline or white aerial hyphae that turned gray or olive in approximately nine days on gPNA (Figure 3). Pigmentation occurred later in colonies on MEA, within 12 days, and was generally lighter (Figure 4). Notably, some colonies on gPNA appear to release a compound that turned the medium around the colonies an orange-red (Figure 3). Similar results were observed on gPNA plates prepared from *P. elliotti* and *P. strobus* needles. *Lecanosticta acicola* colonies grew radially at a rate of 2.5-3 mm per week, as previously reported (CABI). Very little contamination was observed, and what appeared was easily distinguished from *Lecanosticta* by its faster growth or yeast-like colony morphology.

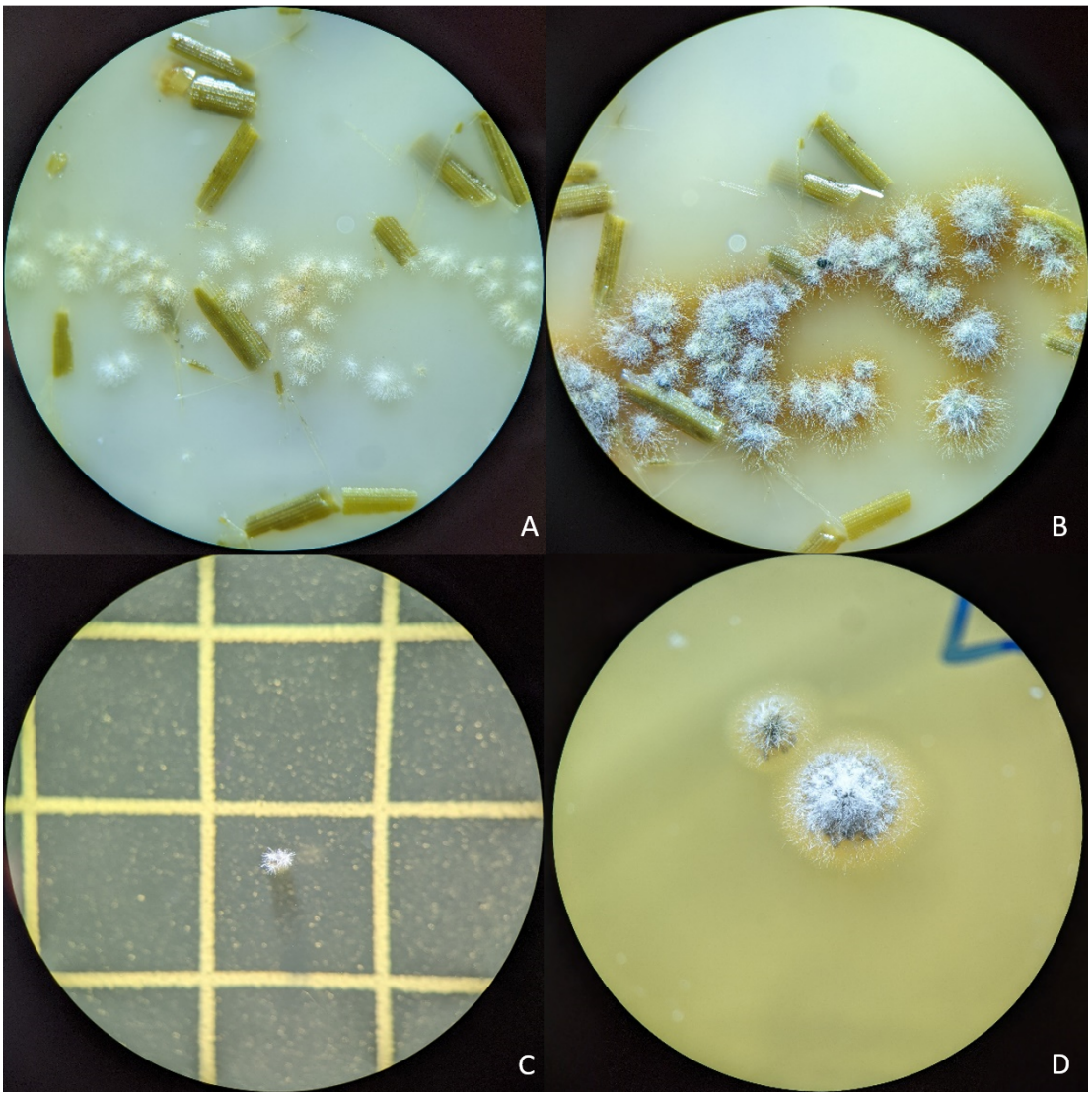

Figure 3. *Lecanosticta acicola* colonies on gPNA at 5 days (A) and 8 days (B), highlighting colony pigmentation and discoloration of the surrounding media, and colonies on MEA at 4 days (C) and 12 days (D).