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Genetic Characterization and Bioclimatic Modeling of the Three Varieties of *Leptographium wagneri* (Cause of Black Stain Root Disease) in the Western USA

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Background

- Black stain root disease (BSRD) is caused by three varieties of *Leptographium wagneri*: var. *wagneri*, var. *ponderosum*, and var. *pseudotsugae*.

1) The current distribution maps of *L. wagneri* are inferred based on host preference of each variety.

→ **Previously reported locations are not verified by DNA-based methods.**

2) *Leptographium wagneri* have a wide range of favorable environmental conditions, and their host could become more susceptible under projected future climates.

→ **It is important to predict *L. wagneri* distributions under current and future climates to minimize damage from BSRD.**

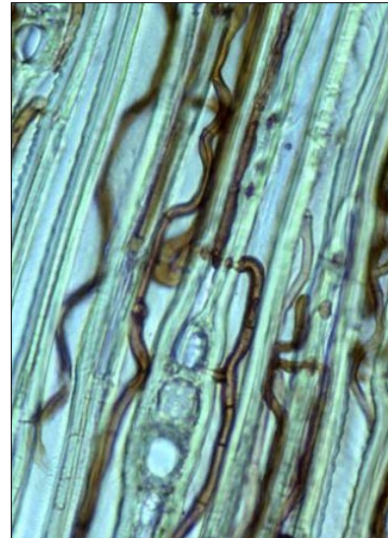


Figure 1. Hyphae of *Leptographium wagneri* in tracheids of Douglas-fir (*Pseudotsuga menziesii*)¹



Figure 2. Staining caused by *Leptographium wagneri* in Douglas-fir (*Pseudotsuga menziesii*)

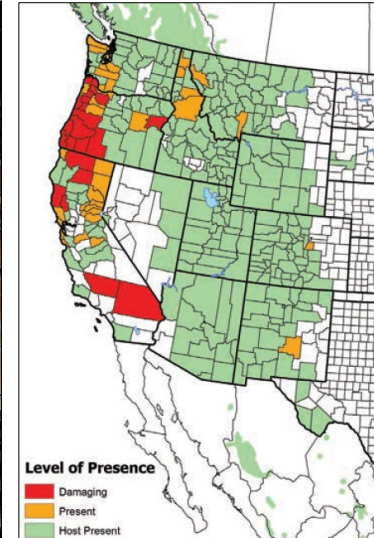


Figure 3. Distribution of hosts and damage from *Leptographium wagneri* var. *pseudotsugae* in western USA²

Expected Results

- Leptographium wagneri* varieties will be characterized using DNA-based methods.
- Previously reported areas of BSRD will be verified with the DNA-based methods.
- Bioclimatic models will predict climatically well-suited geographical areas of BSRD pathogens under present and future climate scenarios.

Significance

The results from this study will provide critical information for developing management strategies to **minimize damage from BSRD**. It will guide land managers as to which areas are, or will become, prone to BSRD pathogens so that less susceptible forest species can be planted or selected for regeneration in forest sites in the western USA.

References

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Objectives

- Characterize three varieties of *L. wagneri*** collected from western USA using DNA-based methods.
- Predict potential geographic distributions of the *L. wagneri*** varieties under contemporary and future climate scenarios using Maximum Entropy bioclimatic modeling.

Methods

1. Sample collections

Hosts with symptoms from reported areas will be selected. For each host, wooden samples (2.5 cm diameter x 5 mm), precise GPS location, and associated host data will be recorded.

2. Fungal isolation & DNA sequencing

For DNA extraction, hyphal tip-derived culture isolates will be subcultured on sterile nylon filters overlaid on Malt Extract Agar media at 15 °C in darkness for 1-2 weeks. PCR will be used to amplify four genetic loci for DNA sequencing.

3. Phylogenetic analyses

Sequences will be edited and aligned with genetic software. Resulting sequences will be compared with reference sequences in GenBank. Phylogenetic analyses will be based on four loci of *Leptographium* isolates.

4. Bioclimate modeling

Maximum Entropy will be used to determine which climatic factors contribute to the occurrence of *L. wagneri* and/or its varieties. Potential distribution of BSRD will be predicted for areas where the pathogen is climatically well-adapted.