

Species-level recognition of the three varieties of *Leptographium wageneri* (cause of black stain root disease) and prediction of their potential suitable climate spaces in the western USA

Daram Choi^{1,5*}, Thomas C. Harrington², David C. Shaw¹, Jane E. Stewart³, Patrick I. Bennett⁴, Ned B. Klopfenstein⁴, Duncan R. Kroese⁵, and Mee-Sook Kim⁵

Black stain root disease (BSRD), a vascular wilt disease of conifers caused by a native insect-vectored fungus, is among the top five most damaging root diseases in forests of the western United States (Hadfield *et al.*, 1986). Previously, the fungi that cause BSRD were described as three varieties of *Leptographium wageneri* — *L. wageneri* var. *wageneri*, *L. wageneri* var. *ponderosum*, and *L. wageneri* var. *pseudotsugae* (Kendrick) Wingfield (Kendrick, 1962; Wingfield, 1985). These *L. wageneri* varieties were differentiated based on morphology, physiology (Harrington and Cobb, 1987), and ecology including host associations, as follows: 1) *L. wageneri* var. *wageneri* on single-leaf pinyon (*Pinus monophylla*) and pinyon pine (*P. edulis*), 2) *L. wageneri* var. *ponderosum* on Jeffrey pine (*P. jeffreyi*) and ponderosa pine (*P. ponderosa*), and 3) *L. wageneri* var. *pseudotsugae* on Douglas-fir (*Pseudotsuga menziesii*) (Harrington and Cobb, 1984, 1986; Hessburg *et al.*, 1995) (Figure 1). The three pathogens were originally classified as different varieties because their distinguishing characteristics were regarded as relatively minor and early DNA-based characterizations were not definitive, but robust phylogenetic analyses can provide the basis for species-level recognition of the *L. wageneri* varieties (Choi *et al.*, 2023). In addition, bioclimatic models may be useful for predicting the suitable climate space (potential distribution) and adaptability for each BSRD pathogen in western North America under the contemporary and projected future climates.

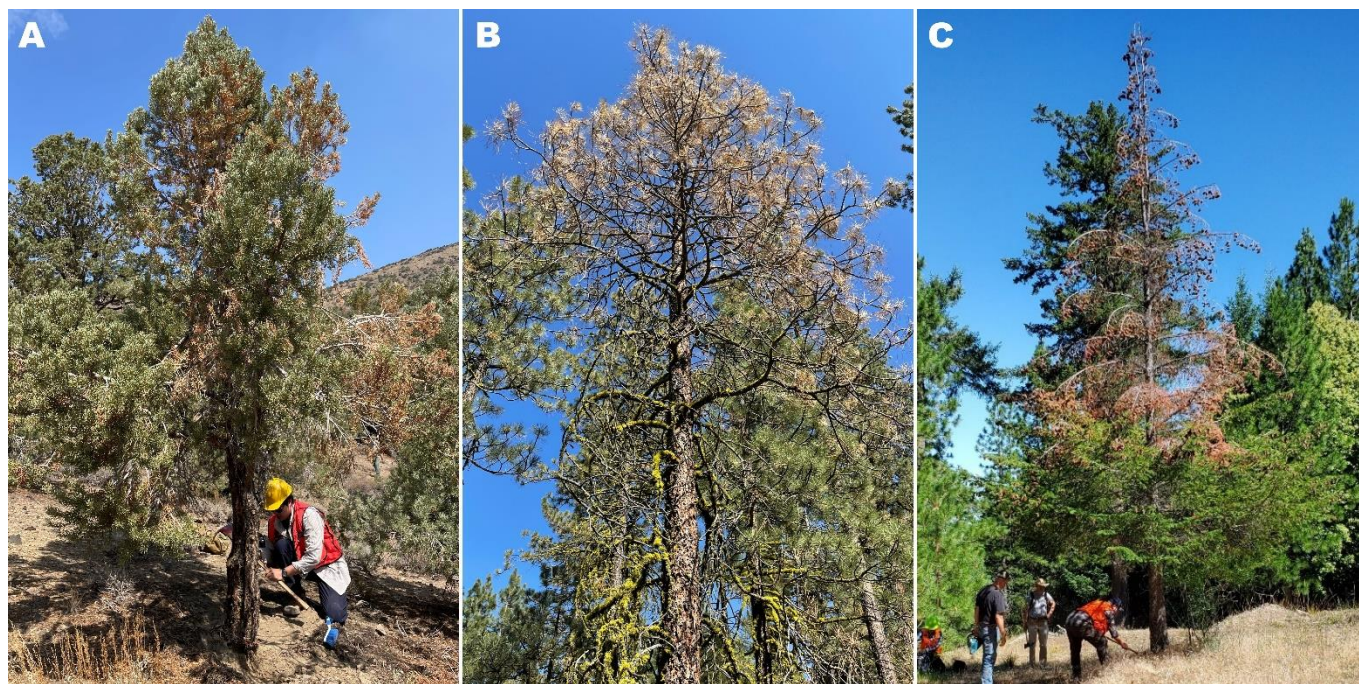


Figure 1: *Leptographium wageneri sensu lato* infected stands show typical crown symptoms such as needle discoloration, needle loss, and tufted needles. Symptoms are showing on (A) a single-leaf pinyon pine (*Pinus*

¹Oregon State University, Department of Forest Engineering, Resources & Management; Corvallis, OR, USA

²Iowa State University, Department of Plant Pathology, Entomology and Microbiology; Ames, IA, USA

³Colorado State University, Department of Agriculture Biology; Fort Collins, CO, USA

⁴USDA Forest Service, Rocky Mountain Research Station; Moscow, ID, USA

⁵USDA Forest Service, Pacific Northwest Research Station; Corvallis, OR, USA

*daram0924@gmail.com

monophylla) infected with *L. wagneri sensu stricto*, (B) ponderosa pine (*P. ponderosa*) infected with *L. ponderosum*, and (C) Douglas-fir (*Pseudotsuga menziesii*) infected with *L. pseudotsugae* (Choi *et al.*, 2023).

The objectives of this study are to 1) genetically identify and determine the phylogenetic relationships among the three species (formerly known as varieties) of BSRD pathogens gathered from the western USA, and 2) predict suitable climate space for BSRD pathogens under present and future climate scenarios using Maximum Entropy (MaxEnt) bioclimate modeling.

BSRD samples were collected from several areas where BSRD was previously reported in Oregon and California. The stained-wood samples were punched from black- to brown-streaked areas of xylem from the root collars of symptomatic trees (*P. monophylla*, *P. ponderosa*, *P. jeffreyi*, and/or *Pseudotsuga menziesii*) (Figure 2). Occurrence data (GPS points, host species, DBH, etc.) were recorded for each sample. The samples were processed for fungal isolation and incubated on selective culture medium to obtain pure cultures (Figure 3). DNA was extracted from 39 *L. wagneri sensu lato* isolates, and 10 different loci (28S large subunit ribosomal, actin, β -tubulin, calmodulin, translation elongation factor 1-alpha, mating-type gene 1-1-3, RNA polymerase II subunit, glyceraldehyde-3-phosphate dehydrogenase, and chitin synthase) were amplified with PCR and the amplicons were used for DNA sequencing.



Figure 2: Black stain streaking appears in sapwood in lower stems of (A) and (B) a single-leaf pinyon (*Pinus monophylla*), and (C) and (D) ponderosa pine (*P. ponderosa*).

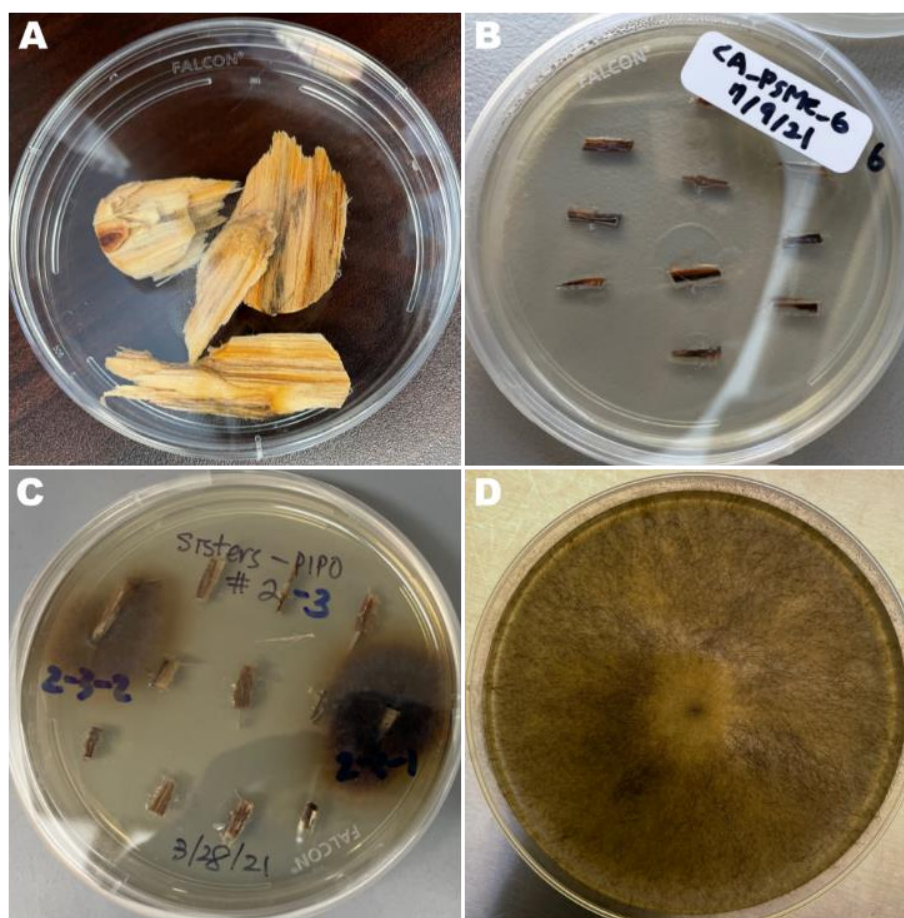


Figure 3: Process for isolating *Leptographium wageneri sensu lato* from collected field samples. (A) Wood samples with stain, (B) embedded wood pieces (ca. 10 x 2.5 x 2.5 mm) on selective CSMA medium, (C) the growth of dark-brown to black-colored hyphae, and (D) the growth of a pure culture derived from hyphal tip isolation (Choi *et al.*, 2023).

Phylogenetic relationships among the three BSRD pathogens were analyzed using maximum likelihood and Bayesian inference of the 10 loci as 1) individual locus, 2) two- and three- loci combinations selected on the basis of parsimony-informative sites, 3) concatenated sequences of all 10 loci, and 4) two-loci combination with multiple outgroups based on phylogenetic analyses from De Beer *et al.* (2022). The results clearly showed that the separation of the three BSRD pathogens (formerly recognized as varieties of *L. wageneri*) into distinct species is well-supported. This genetic evidence combined with previously recognized differences in morphological, physiological, and ecological characteristics strongly supported the species-level recognition of the three BSRD pathogens, as follows: *L. wageneri* for *L. wageneri* var. *wageneri*, *L. ponderosum* for *L. wageneri* var. *ponderosum*, and *L. pseudotsugae* for *L. wageneri* var. *pseudotsugae* (Choi *et al.*, 2023).

Occurrence data were used to predict suitable climate space for BSRD pathogens under contemporary (1970-2000) and future (2041-2060 and 2081-2100) climate scenarios (SSP2-4.5 and SSP5-8.5) with 19 bioclimatic variables at 30-second (~1 km²) spatial resolution from WorldClim. Three separate models were developed using MaxEnt with DNA-confirmed pathogen occurrence points; *L. wageneri* (N=10), *L. ponderosum* (N=10), and *L. pseudotsugae* (N=38). AUC values of the three bioclimate models ranged from 0.88-0.97, which are considered as reliable predictions. For the *L. wageneri* models, the potential distributions are similar in contemporary and future climates, which predict areas with high probability of suitable climate in the Oregon Cascades and northern Sierra Nevada Mountains, though the host trees (pinyons) do not presently occur in these areas. For the *L. ponderosum*

models, California and Oregon contain areas with high probability of suitable climate in all time frames, while the suitable spaces shift northward under the future climate scenarios. For the *L. pseudotsugae* models, the suitable climate spaces are concentrated in the regions adjacent to Pacific Ocean under the contemporary climates, while the suitable climate spaces extend inland under projected future climates. These predictions were produced based on BSRD occurrence, but more reliable predictions might be obtained by comparisons with the potential distributions of each pathogen's host species and this work is underway. Nevertheless, these data offer useful information about present or potentially vulnerable areas to BSRD pathogens that can guide land managers' decisions regarding forest planting and regeneration in targeted areas of the western USA.

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Compiled by:

Rachel K. Brooks
Washington State Department of Natural Resources
Forest Resilience Division
Olympia, Washington

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