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Pathogenicity and Virulence of *Pythium* Species Obtained from Forest Nursery Soils on Douglas-Fir Seedlings

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Abstract

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Pythium species are common soilborne oomycetes that occur in forest nursery soils throughout the United States. Numerous species have been described from nursery soils. However, with the exception of *P. aphanidermatum*, *P. irregulare*, *P. sylvaticum*, and *P. ultimum*, little is known about the potential for other *Pythium* species found in nursery soils to cause damping-off of tree seedlings. A greenhouse study was conducted to evaluate the pathogenicity and virulence of 44 *Pythium* isolates representing 16 species that were originally recovered from soil at three forest nurseries in Washington and Oregon. Seeds of Douglas-fir (*Pseudotsuga menziesii*) were planted into soil infested with each of the isolates. Seedling survival, the number of surviving seedlings with necrotic root lesions, and taproot length were evaluated 4 weeks later. Responses of Douglas-fir to inoculation varied significantly depending on *Pythium* species and isolate. Eight species (*P. dissotocum*, *P. irregulare*, *P. aff. macrosporum*, *P. mamillatum*, *P. aff. oopapillum*, *P. rostratifingens*, *P. sylvaticum*, and *P. ultimum* var. *ultimum*) significantly reduced the number of surviving seedlings compared to the noninoculated treatment. However, all *Pythium* species caused a greater percentage of seedlings to develop root lesions (total mean 40%) than was observed from noninoculated seedlings (17%). Taproot length varied little among *Pythium* treatments and was not a useful character for evaluating pathogenicity. Results confirm the ability of *P. irregulare*, *P. mamillatum*, and *P. ultimum* var. *ultimum* to cause damping-off of Douglas-fir seedlings, and are indicative that other species such as *P. dissotocum*, *P. aff. macrosporum*, *P. aff. oopapillum*, *P. rostratifingens*, and *P. sylvaticum* may also be responsible for seedling loss.

Approximately 200 million tree seedlings are produced annually by forest nurseries located in the western United States (California, Idaho, Montana, Oregon, and Washington) (1). The majority of the seedlings are sold as 1- or 2-year-old bareroot conifer seedlings and are used for reforestation, although some seedlings are sold to Christmas tree and ornamental nursery operations. Douglas-fir (*Pseudotsuga menziesii*) is the most commonly planted species and accounts for approximately 75 million of the seedlings sold by forest nurseries in the region (18).

Pythium species are frequently associated with damping-off of forest nursery seedlings and are considered one of the most important soilborne pathogens limiting seedling production in the Pacific Northwest (PNW) region of the United States (Idaho, Oregon, and Washington) and Canada (1,12). Unfortunately, relatively little is known regarding the identity of regional *Pythium* species capable of causing disease. Four species have been commonly found in forest nurseries of the PNW: *P. aphanidermatum*, *P. irregulare*, *P. mamillatum*, and *P. ultimum* (2,4). However, at least 15 other species are also present in nursery soils (18), and it is unknown whether any or all of these species can also cause damping-off.

Management of soilborne pests and pathogens in bareroot forest nurseries of the PNW of the United States is primarily achieved by preplant fumigation with methyl bromide or dazomet (3,6,19). Supplemental control is provided by periodic application of fungicides, such as mefenoxam. Forest nurseries may survey field soils prior to planting in order to determine whether damaging levels of soilborne pathogens are present and to decide if fumigation is necessary. In the case of *Pythium*, populations exceeding 100 propagules per gram of soil (ppg) are considered problematic (1,7). However, it is unknown whether all *Pythium* species present in regional forest nursery soils are pathogenic to forest tree seedlings, or whether some proportion of the *Pythium* community is nonpathogenic (1,3). Therefore, decisions to fumigate based on population counts alone may overestimate the amount of risk if nonpathogenic isolates are abundant, thereby resulting in the unnecessary application of fumigants and fungicides. If forest nursery managers could identify relative numbers of pathogenic and nonpathogenic *Pythium* species, they could better evaluate the need for treatment. The first step toward this goal is to identify those *Pythium* species present in the soil that are pathogenic to forest nursery seedlings and to determine if variation exists among isolates within *Pythium* species for pathogenicity and virulence.

In order to determine pathogenicity and evaluate the relative virulence of *Pythium* species and isolates found in forest nurseries, a greenhouse study was conducted using 44 *Pythium* isolates representing 16 *Pythium* species. These isolates were originally collected in 2008 during a survey of three forest nurseries (two in Oregon, one in Washington) to determine the diversity of *Pythium* species in nursery soils (18). The objective of this research was to assess the potential of *Pythium* species and isolates to reduce Douglas-fir seedling survival, cause root lesions, and reduce taproot length.

Materials and Methods

Isolate selection. Forty-four *Pythium* isolates were selected from the isolate collection of a previous survey (18) to represent the range of *Pythium* species obtained from the soils of three forest nurseries in Washington and Oregon (Table 1). Whenever possible, isolates that were representative of a particular species were also chosen to originate from each of the three nurseries in order to

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