

# Understanding the Pathology of Douglas-fir Seedlings in Pacific Northwest Nurseries

Jerry Weiland

**Jerry Weiland**, Research Plant Pathologist, USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave, Corvallis, OR 97330; E-mail: [Jerry.Weiland@ars.usda.gov](mailto:Jerry.Weiland@ars.usda.gov).

Weiland J. 2014. Understanding the Pathology of Douglas-fir Seedlings in Pacific Northwest Nurseries. In: Wilkinson KM, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2013. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-72. 38-40. Available at: [http://www.fs.fed.us/rm/pubs/rmrs\\_p072.html](http://www.fs.fed.us/rm/pubs/rmrs_p072.html)

**Abstract:** Douglas-fir seedlings are infected by a number of *Pythium* species causing damping-off and root rot. As soil fumigation continues to be more tightly regulated, knowledge about the identity and pathogenicity of *Pythium* species in forest nurseries will be increasingly important for studies that evaluate the effectiveness of nonfumigant disease control measures, including fungicide and biological control efficacy trials. The diversity of *Pythium* species at three Pacific Northwest (PNW) forest nurseries was evaluated and each nursery was found to have a unique assemblage of species. Furthermore, each *Pythium* species was found to cause a different level of disease on Douglas-fir seedlings. Subsequent fungicide and biological control efficacy studies found that differences in species responses to mefenoxam, fosetyl-AI, and *Streptomyces lydicus*, a biological control agent.

**Key Words:** *Pythium*, forest nursery, fungicide, biological control, *Streptomyces lydicus*, mefenoxam, fosetyl-AI

## Introduction

Forest nurseries in the Pacific Northwest (PNW, states of ID, OR, and WA) are affected by a number of soilborne pathogens including *Pythium* species. However, there is limited information regarding the identity of *Pythium* species affecting seedling production or the amount of disease (damping-off, root lesions, and root rot) that each *Pythium* species can cause. Soilborne diseases are primarily managed by soil fumigation with a combination of methyl bromide and chloropicrin, or with dazomet (Weiland and others 2013a). Supplemental disease control is also provided with applications of mefenoxam or phosphonate fungicides (fosetyl-AI and phosphorous acid). However, soil fumigant use has become increasingly difficult with increasing state and federal regulation, and growers may need to increasingly rely on alternative disease control methods to obtain adequate disease control. Currently, it is unknown how *Pythium* species of PNW forest nurseries respond to two of the most commonly used fungicides, mefenoxam and fosetyl-AI. It is also unknown how the different *Pythium* species from forest nurseries respond to biological control agents. Our research objectives were therefore to: (1) evaluate *Pythium* species diversity in PNW forest nurseries; (2) determine the pathogenicity of different *Pythium* species on Douglas-fir seedlings; and, (3) evaluate the sensitivity of different *Pythium* species to mefenoxam, fosetyl-AI, and the biological control agent, *Streptomyces lydicus*. *Streptomyces lydicus* is a commercially available, antibiotic-producing bacterium that suppresses damping-off and root rot fungi, including *Pythium* species.

## Materials and Methods

*Pythium* species were sampled from three forest nurseries (two in Oregon, one in Washington) by baiting soil samples with rhododendron leaf disks and Douglas-fir needles, and by dilution plating (Weiland 2011). Nursery A was located in southwestern Washington, Nurseries B and C were located in northwestern Oregon. Species were identified by morphology and by DNA sequence analyses. Sixteen *Pythium* species were then used to inoculate Douglas-fir seedlings in a greenhouse study (Weiland and others 2013b) with noninoculated seedlings used as a negative control. The number of seedlings that were killed and the number of seedlings with root lesions were then recorded for each species tested. Species of *P. irregulare*, *P. sylvaticum*, and *P. ultimum* were also tested against concentrations of mefenoxam (0.1-100 µg/ml) and fosetyl-Al (1-2500 µg/ml) on fungicide-amended V8 media in petri plates. The effective concentration of each fungicide required to reduce diameter growth by 50% (EC50) was calculated and compared among the three species to determine their sensitivity to each fungicide. Finally, inhibition of 16 *Pythium* species was measured in dual-culture plates containing *Streptomyces lydicus* strain WYEC 108. The minimum distance at which the growth of the *Pythium* isolate towards the *S. lydicus* culture stopped (inhibition zone distance) was recorded and used to determine the sensitivity of each *Pythium* species to the biological control agent.

## Results and Discussion

Nineteen *Pythium* species were identified from the three forest nurseries, of which at least eleven had not been previously been described

from forest nursery soils (table 1). Each nursery was associated with a different community of *Pythium* species, with *P. irregulare* the most common species at nursery A, *P. 'vipa'* the most common at nursery B, and *P. dissotocum* the most common at nursery C (table 1).

Each of the 16 *Pythium* species tested was able to cause disease (figure 1). Eight species (*P. mamillatum*, *P. rostratiformis*, *P. aff. oopapillum*, *P. dissotocum*, *P. sylvaticum*, *P. ultimum*, *P. aff. macrosporum*, and *P. irregulare*) reduced survival of Douglas-fir seedlings by at least 25% and were considered highly virulent (aggressive) species. Although the remaining species reduced seedling survival by less than 25%, these species did cause significantly more root lesions than were observed on the noninoculated seedlings, and were therefore considered weakly virulent species.

In the fungicide and biological control sensitivity studies, isolates of *P. irregulare* were generally found to be less sensitive to mefenoxam than isolates of either *P. sylvaticum* or *P. ultimum* (0.02 µg/ml for *P. irregulare* versus 0.06 µg/ml for both *P. sylvaticum* and *P. ultimum*). However, two isolates of *P. ultimum* (one each from nursery B and C) were found that were 5000-6000 times more resistant to mefenoxam than any of the other isolates tested. No differences were observed in the sensitivity of *P. irregulare*, *P. sylvaticum*, or *P. ultimum* isolates to fosetyl-Al (1256-1508 µg/ml). Finally, *Pythium* species were also found to vary in sensitivity to the biological control agent *Streptomyces lydicus*. Inhibition ranged from 17 mm (*P. aff. oopapillum*) up to 34 mm (*P. aff. mercuriale*).

The diversity of *Pythium* species in forest nursery soils was much greater than previously characterized (Hansen and others 1990, James 2002) and was observed to vary from nursery to nursery. This diversity is reflected in the ability of individual species to cause disease and to be controlled by fungicides and biological control agents.

**Table 1.** Frequency of *Pythium* species from soil at three forest nurseries.

Species	Nursery A (WA)	Nursery B (OR)	Nursery C (OR)	Total
<i>P. aphanidermatum</i>	0	0	1	1
<i>P. aff. attrantheridium</i>	1	1	0	2
<i>P. dissotocum</i>	6	0	140	146
<i>P. irregulare</i>	194	29	17	240
<i>P. irregulare</i> group III	17	0	0	17
<i>P. irregulare</i> group IV	2	10	0	12
<i>P. aff. macrosporum</i>	19	46	20	85
<i>P. mamillatum</i>	3	0	7	10
<i>P. aff. mercuriale</i>	0	0	1	1
<i>P. middletonii</i>	3	0	0	3
<i>P. aff. oopapillum</i>	0	0	2	2
<i>P. pachycaule</i>	1	1	0	2
<i>P. rostratiformis</i>	2	1	0	3
<i>P. aff. rostratum</i>	0	0	2	2
<i>P. aff. spiculum</i>	16	2	33	51
<i>P. sylvaticum</i>	0	27	25	52
<i>P. torulosum</i>	31	0	10	41
<i>P. ultimum</i>	2	23	41	66
<i>P. 'vipa'</i>	3	160	1	164
Total isolates	300	300	300	900
Total species	14	10	13	19

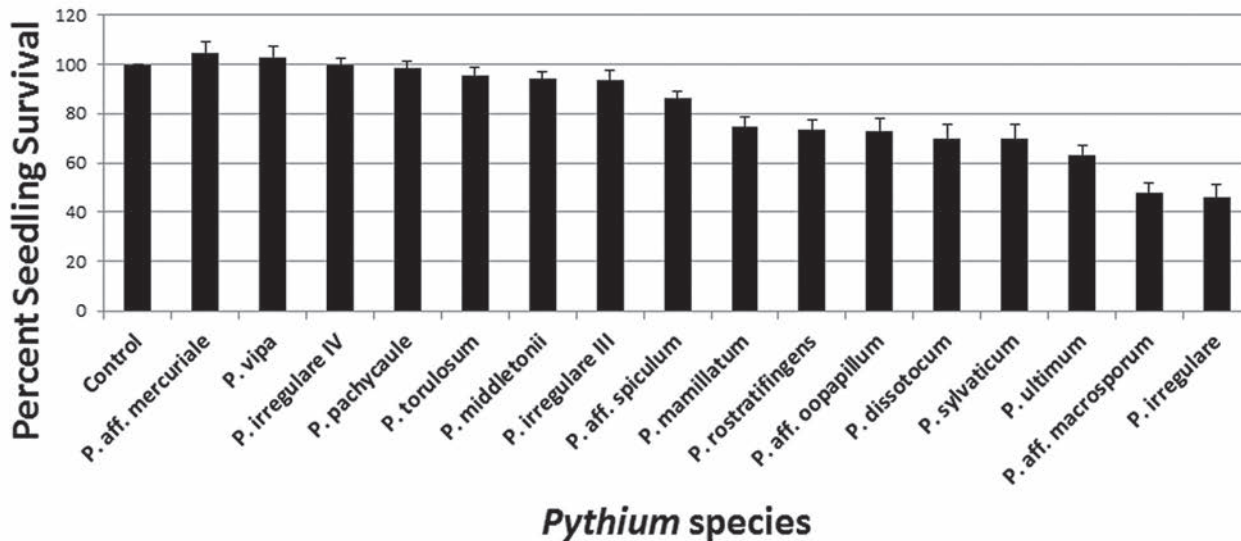


Figure 1. Percent survival of seedlings inoculated with 16 different *Pythium* species.

## Summary

Many *Pythium* species are found in PNW forest nurseries and each nursery has a unique assemblage of species. In turn, each *Pythium* species causes a different amount of disease to Douglas-fir seedlings. As a consequence, the amount of *Pythium* damping-off at each nursery will likely be influenced by the predominant *Pythium* species that occur at each nursery. Nurseries with high populations of aggressive *Pythium* species would be expected to experience more damage than those with mostly weakly-pathogenic *Pythium* species. In addition, *Pythium* species vary in sensitivity to mefenoxam and *S. lydicus*, but not to fosetyl-AI. As regulations and costs associated with soil fumigation continue to increase, emphasis will need to be placed on integrated pest management practices that target multiple soilborne pathogen species.

## References

- Hansen, EM, Myrold DD, Hamm PB. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. *Phytopathology* 80: 698-704.
- James RL. 2002. Effects of spring applications of dazomet on root diseases and performance of Douglas-fir and western white pine transplants. USDA Forest Service Nursery, Coeur d'Alene, Idaho. Forest Health Protection Report 02-9.
- Weiland JE. 2011. Influence of isolation method on recovery of *Pythium* species from forest nursery soils in Oregon and Washington. *Plant Disease* 95: 547-553.
- Weiland JE, Littke WR, Haase DL. 2013a. Forest nurseries face critical choices with the loss of methyl bromide fumigation. *California Agriculture* 67: 153-161.
- Weiland JE, Beck BR, Davis A. 2013b. Pathogenicity and virulence of *Pythium* species obtained from forest nursery soils on Douglas-fir seedlings. *Plant Disease* 97: 744-748.