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# ROOT DISEASE OF 1-0 BAREROOT DOUGLAS-FIR SEEDLINGS – USDA FOREST SERVICE LUCKY PEAK NURSERY, BOISE, IDAHO

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## ABSTRACT

Douglas-fir 1-0 seedlings at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho were affected by root disease during the 2000 growing season. Isolations from roots of diseased seedlings and assay of soil within diseased areas revealed that *Fusarium oxysporum* and two species of *Phytophthora* (*P. cactorum* and *P. pseudotsugae*) were primarily associated with the disease. Disease pattern within affected beds indicated that *Phytophthora* spp. were important contributors to disease intensity. Relatively high disease losses occurred despite pre-plant soil fumigation with methyl bromide/chloropicrin. Douglas-fir is very susceptible to root-pathogenic fungi and may be significantly stressed during warm summer periods at the nursery. Therefore, this conifer species may best be grown at other nurseries where it will not be overly stressed and damaged by root pathogens.

## INTRODUCTION

During the late spring of 2000, growers at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho noticed portions of beds of bareroot 1-0 Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco) that had extensive seedling mortality. The most severe disease was localized causing groups of dead and dying seedlings (figure 1). Several disease groups were within low portions of beds where water had accumulated and remained for prolonged periods. Seedlings within and adjacent to disease groups exhibited typical root disease symptoms, i.e.,

initially they turned chlorotic followed by development of foliar necrosis characterized by reddish-brown needles. After seedlings were killed, they lost their foliage and typically the only indication of a dead seedling was presence of a blackened main stem. Analyses were conducted to elucidate probable causes of the disease in order to provide growers with alternatives to reduce future disease losses.

Figure 1. Large root disease area in 1-0 Douglas-fir seedlings at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

## MATERIALS AND METHODS

Several seedlings with various levels of disease symptoms were carefully extracted from the soil and transported to the laboratory for analysis of associated, potentially-pathogenic organisms. Sampled seedlings were washed thoroughly to remove soil particles, and their roots (consisting mostly of a main tap root) cut into pieces about 2-3 mm in length. Root pieces were surfaced sterilized in 0.5% aqueous sodium hypochlorite, rinsed in sterile, distilled water and placed on two selective agar media. One medium was selective for *Fusarium* spp. and closely-associated

organisms (Komada 1975) and the other medium consisted of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene which was selective for water mold fungi, particularly those in the genera *Pythium* and *Phytophthora* (James et al. 1990, 1996). Plates with Komada's medium were incubated 5-7 days at about 24°C under diurnal cycles of cool, fluorescent light. Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification using the taxonomy of Nelson and others (1983). Plates of V-8 juice agar were incubated in the dark at about 24°C for 3 days. Water mold fungi were transferred to new V-8 juice agar, PDA and water agar (WA) slants for identification using the taxonomy of Stamps et al. (1990) and Waterhouse (1956, 1968). To facilitate identification, slants inoculated with water mold fungi were flooded with non-sterilized pond water three days after inoculation.

To estimate soil populations of potentially-pathogenic *Fusarium* and water mold fungi and potentially-antagonistic *Trichoderma* spp. within a representative disease area, five soil samples were collected at approximately equidistant locations along a transect in the middle of the seedbed. At each sample point a soil core was taken to a depth of about 20 cm. Soil was placed into plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard dilutions (James 2000a; James and Beall 1999; James et al. 1990, 1996) were conducted on the soil samples. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven-dried at about 100°C for at least 24 hours until sample weight had stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3 percent WA and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated as described above. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations, expressed as number of colony-forming units (cfu) per g of oven-dried soil, were calculated. Selected *Fusarium* isolates were identified as described above. For assay of water mold populations, 0.5 g of soil was combined with 10 ml of 0.3 percent WA. One ml of solution was placed on each of three plates containing V-8 juice agar and incubated in the dark as described above. Water mold colonies were identified on the basis of their diameter after 3 days, feathery margin, and growth within rather than superficially on the agar surface. Selected isolates were identified as described above.

*Fusarium*, *Trichoderma*, and water mold populations were determined for each sample point. Averages were calculated and the ratio of *Trichoderma* to *Fusarium* populations (T/F ratio) was calculated for each sample. This ratio may be useful as an approximation of potential disease suppressiveness in nursery soils (James 1998; James et al. 1996). Generally, the higher the ratio, the less potential for *Fusarium*-caused

disease due to expected anagonism by *Trichoderma* spp.

## RESULTS AND DISCUSSION

Root isolations from seedlings displaying disease symptoms yielded four groups of potentially-pathogenic fungi (table 1). The most commonly-associated group was *Fusarium*, comprised exclusively of *F. oxysporum* Schlecht. This fungal species was isolated from all sampled seedlings and more than 83% of the sampled root pieces (approximate root system colonization rate). The second most common group of potential pathogens was *Phytophthora*, which was isolated from all but one of the sampled seedlings and colonized nearly half of the sampled root systems. Two species of *Phytophthora* were isolated. The most common was *P. cactorum* (Leb. and Cohn.) Schr.; *P. pseudotsugae* Hamm and Hansen was isolated much less frequently. *Pythium* spp. were isolated from 60% of the seedlings and nearly 1/3 of the sampled root pieces. The most frequently-isolated *Pythium* spp. was *P. irregulare* Buisman; *P. ultimum* Trow. and *P. aphanidermatum* (Edson) Fitzp. were also isolated, although at much lower levels. The final group of potential pathogens consisted of *Cylindrocarpon destructans* (Zins.) Scholten, which was isolated from 40% of the seedlings and just over 15% of the sampled root systems.

Soil samples from within a severe disease area revealed relatively high populations of *Fusarium* (table 2). All isolates recovered from soil samples were identified as *F. oxysporum*. Populations of *Phytophtora* (primarily *P. cactorum*) were also isolated at relatively high levels with 3 of the 5 samples exceeding the 100 cfu/g threshold usually associated with potential disease problems (Hildebrand and Dinkel 1988; James 2000b). *Pythium* spp. were either absent from soil samples or found at very low levels.

High soil populations of *Trichoderma* spp. may sometimes suppress soilborne pathogens due to their potential antagonism toward pathogens (Papavizas 1985; Papavizas and Lumsden 1980). The average ratio of *Trichoderma* to *Fusarium* (T/R ratio) exceeded 1.0 (table 2), indicating that there were generally higher levels of *Trichoderma* than *Fusarium* in the soil. Generally, the higher the ratio, the greater the potential buffering of *Trichoderma* spp. to reduce *Fusarium*-associated disease (James 1998; James et al. 1996). In two of the 5 samples, the T/R ratio was relatively high. In the other 3 samples, the ratio was either below or slightly above 1.0. Also, *Trichoderma* spp. were recovered at quite low levels from diseased seedling roots (table 1). Therefore, *Trichoderma* spp. were probably present at insufficient levels to successfully counter the high *Fusarium* and *Phytophthora* populations in soil.

*Fusarium oxysporum* is a very common soil-inhabiting fungus at the Lucky Peak Nursery (James and Beall 1999, 2000). This species is comprised of both pathogenic and non-pathogenic strains (Gordon and Martyn 1997; Gordon and Okamoto 1992a, 1992b) and frequently isolated from the roots of both diseased and healthy-appearing bareroot seedlings (James and Gilligan 1988; James et al.

1991). Soil populations at a nursery tend to vary widely and appear related to recent cropping history and length of time fields have been fallowed (James 2000a; James and Beall 2000; James et al. 1996). In general, *F. oxysporum*

Table 1. Colonization of 1-0 Douglas-fir seedling roots by selected potentially-pathogenic and antagonistic fungi within bareroot production beds at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

| Percent Root Colonization[1] | | | | | |
|---|---|---|---|---|---|
| Seedling No. | *Fusarium* | *Cylindrocarpon* | *Trichoderma* | *Pythium* | *Phytophthora* |
| 1 | 100 | 0 | 0 | 60 | 20 |
| 2 | 75 | 38 | 0 | 100 | 0 |
| 3 | 63 | 38 | 0 | 0 | 80 |
| 4 | 100 | 0 | 33 | 0 | 60 |
| 5 | 100 | 0 | 0 | 0 | 80 |
| 6 | 67 | 55 | 22 | 0 | 40 |
| 7 | 100 | 0 | 0 | 20 | 80 |
| 8 | 100 | 0 | 0 | 80 | 20 |
| 9 | 100 | 0 | 0 | 40 | 60 |
| 10 | 63 | 13 | 25 | 20 | 40 |
| Average | 83.1 | 15.6 | 6.5 | 32.0 | 48.0 |

[1] Based on colonization of root pieces (maximum of 10 sampled per seedling) colonized with appropriate fungus.

Table 2. Soil populations of selected pathogenic and antagonistic fungi within a root disease area located within 1-0 Douglas-fir bareroot production beds at the USDA Forest Service Lucky Peak Nursery, Boise Idaho.

| Colony-Forming Units per Gram of Oven-dried Soil | | | | | |
|---|---|---|---|---|---|
| Sample No. | *Fusarium* | *Trichoderma* | T/F Ratio[1] | *Pythium* | *Phytophthora* |
| 1 | 404 | 2357 | 5.83 | 0 | 7 |
| 2 | 2036 | 3053 | 1.50 | 0 | 136 |
| 3 | 2229 | 3242 | 1.45 | 0 | 54 |
| 4 | 2691 | 1413 | 0.52 | 0 | 121 |
| 5 | 681 | 2794 | 4.10 | 7 | 109 |
| Average | 1608 | 2572 | 1.60 | 1.4 | 85.4 |

[1] Ratio of *Trichoderma* to *Fusarium* populations.

remains viable in soil because it forms long-lived resting structures (chlamydospores and sclerotia)(James et al. 1991; Nelson et al. 1983). However, without susceptible host material or organic matter as food sources, soil populations will generally decrease over time (Burgess 1981; James et al. 1991). Soil populations are also greatly reduced or eliminated when soil is fumigated with general biocides such as methyl bromide/chloropicrin (Boyd 1971; Hildebrand and Dinkel 1988; James 1989). Other soil fumigants, such as dazomet, may not be as effective in reducing populations of *F.oxysporum* to sufficient levels to control diseases (Hoffman and Williams 1988; James and Beall 1999). Another problem is that fumigated fields may be easily recolonized by this fungus from adjacent, non-fumigated fields or contaminated equipment and infested seed (Danielson and Davey 1969; James 1987; Vaartaja 1967). Therefore, although fumigation with an effective biocide will usually control soilborne diseases, there is always the chance that disease might occur despite soil fumigation.

*Phytopthora* spp. have previously been found on Douglas-fir seedlings at the Lucky Peak Nursery (James 1997). However, in the past, their impact as disease-causing pathogens has generally been low. Damage from *Phytophthora* has most often been restricted to poorly-drained seedbeds where water accumulates and remains for prolonged periods. *Phytophthora cactorum* has previously been found in northern Idaho on both bareroot seedlings and young tree improvement plantings (James 1993, 2000c). The other *Phytophthora* spp. found in this investigation (*P. pseudotsugae*) has not previously been reported in Idaho, although this species has been recognized on bareroot Douglas-fir seedlings within Oregon and Washington nurseries (Hamm and Hansen 1983).

*Pythium* spp. have not generally been important soil pathogens at the Lucky Peak Nursery (James and Beall 1999). Although these fungi are sometimes isolated from the roots of diseased seedlings and adjacent soil, they are not nearly as important as *Fusarium* spp. at the Lucky Peak Nursery (James and Beall 1999, 2000). The other potentially-pathogenic fungus isolated from diseased seedling roots, *Cylindrocarpon destructans*, is a common rhizosphere inhabitant and colonizer of root cortical tissues (Booth 1966). This fungus is often not as pathogenic as some other soil-inhabiting fungi, such as *Fusarium* and *Phytophthora* spp. (James et al. 1994).

Based on isolation results from both roots of diseased seedlings and soil within disease areas, it was concluded that the disease on 1-0 Douglas-fir seedlings at the Lucky Peak Nursery was due primarily to a combination of *F. oxysporum* and two *Phytophthora* species. Both groups of organisms were found at sufficient levels capable of causing disease. It is possible that the extensive, rapid disease found in affected beds was due to synergistic action by these two groups of pathogens. Disease levels were highest near the end of one bed (figure 1) where water drainage was impeded; *Phytophthora* spp. have traditionally been found causing more problems near the end of seedbeds in conifer seedling nurseries (Hamm and Hansen 1982, 1983; Hansen

et al. 1979). On the other hand, *F. oxysporum* usually causes more dispersed disease throughout affected beds, i.e., affected seedlings are scattered with non-symptomatic seedlings prevalent throughout affected areas (James 1996; James and Beall 2000).

Rather extensive root disease occurred within portions of 1-0 Douglas-fir seedbeds despite pre-plant soil fumigation with methyl bromide and chloropicrin, which is currently the standard treatment at the nursery. Sufficient pathogen inoculum either survived fumigation or was reintroduced following fumigation (Danielson and Davey 1969; Vaartaja 1967). Douglas-fir is notoriously susceptible to several root-pathogenic fungi including both *Fusarium* and *Phytophthora* (Bloomberg 1971, 1973; Hansen et al. 1979; James et al. 1991). As stress increases, seedlings become more susceptible to infection and disease by these fungi. Temperature, moisture, and nutrient stresses probably contribute to disease susceptibility (Bloomberg 1973, 1985; Hamm and Hansen 1982). High spring and summer temperatures experienced at the Lucky Peak Nursery probably contribute to root disease losses, particularly during the first growing season (James 1996, 1997). Young Douglas-fir seedlings may become more stressed by high ambient temperatures compared to other species grown at the nursery, such as ponderosa and lodgepole pine. As a result, disease damage to Douglas-fir may sometimes exceed levels normally found on pine species. One way to possibly reduce future disease losses may be to grow Douglas-fir seedlings at other nurseries where they are not likely to be as stressed. Likewise, it may be beneficial for other nurseries to consider having their pine species grown at the Lucky Peak Nursery. In this way, high-quality, healthy seedlings may be produced within environments best suited to their optimum production.

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