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ROOT DISEASES OF BAREROOT WESTERN LARCH SEEDLINGS – USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Groups of chlorotic and necrotic bareroot western larch seedlings were evident at the USDA Forest Service Nursery, Coeur d'Alene, Idaho at the end of their first growing season in a field that had undergone pre-plant soil fumigation with dazomet (Basamid®). Damage expanded early in the second growing season and was limited to poorly-drained, low portions of seedbeds. Isolations from the roots and rhizosphere soil of symptomatic seedlings consistently yielded species of *Phytophthora*, *Pythium*, and, to a lesser extent, *Fusarium*. Root systems were extensively colonized by *Phytophthora* and *Pythium* spp. The major *Phytophthora* spp. isolated were *P. cactorum* and *P. megasperma*; three species of *Pythium* were isolated: *P. irregulare*, *P. ultimum*, and *P. aphanidermatum*. *Phytophthora* and *Pythium* spp. were considered the major elicitors of root diseases of these western larch seedlings. Management implications are discussed.

INTRODUCTION

Western larch (*Larix occidentalis* Nutt.) is one the major reforestation conifer species in the Northern Region (James et al. 1995). Large numbers of larch seedlings are routinely produced as both container and bareroot stock at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Unfortunately, diseases may sometimes adversely affect larch seedling production. For example, western larch seed may be contaminated with potentially-pathogenic fungi (James 1986c, 1988, 1990a; James et al. 1995, 1996). A major foliar blight pathogen of

container-grown stock is *Botrytis cinerea* Pers. ex. Fr. (James 1984; James and Woo 1984). Root diseases, caused primarily by *Fusarium* spp., may also be important on container-grown stock (James 1986a, 1986b, 1987a, 1989a, 1990b, 1991). On bareroot stock, *Meria laricis* Vuill. is very damaging, causing both growth reduction and mortality during severe disease epidemics (James 1985, 1998; James et al. 1995). Root diseases of bareroot western larch seedlings are rare at the Coeur d'Alene Nursery (James 1993; James et al. 1991). Bareroot seedling root disease is usually effectively controlled by pre-plant soil fumigation with general biocides including methyl bromide/chloropicrin (Boyd 1971) and dazomet (Basamid®) (James 1989b; James et al. 1990).

During the latter part of the 1999 growing season, portions of bareroot 1-0 western larch seedbeds contained seedlings that were smaller, chlorotic, and of lower densities than normal. This condition persisted into the second growing season (figure 1). Portions of beds with diseased seedlings were poorly drained where water often accumulated keeping soils saturated for prolonged periods (figure 2). In order to make the proper recommendations to restrict disease spread and reduce further losses, an evaluation was conducted to quantify associated organisms on diseased seedlings.

MATERIALS AND METHODS

Ten larch seedlings with various levels of disease symptoms were randomly selected within affected seedbeds for laboratory analyses. These seedlings were carefully extracted from the soil, kept refrigerated, and transported to the laboratory. Seedling roots were carefully washed to remove adhering soil particles. On five of the seedlings, samples of rhizosphere soil were collected and stored in sterile, distilled water for further analysis. Seedling roots were dissected into pieces 3-5 mm in length. Root pieces were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite), rinsed in sterile water, and aseptically placed on two selective agar media: the first selective for *Fusarium* spp. and closely-related fungi(Komada 1975) and the other selective for water mold fungi in the genera *Pythium* and *Phytophthora* (V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloro-nitrobenzene (James et al 1991). Plates of Komada's medium, each containing 10 root pieces per sampled seedling, were incubated at about 24°C under diurnal cycles of cool, fluorescent light for 7-10 days. After incubation, fungi were identified to genus based on morphological characteristics; selected *Fusarium* isolates were transferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher et al. 1982) for identification using the taxonomy of Nelson et al. (1983). Plates with V-8 juice agar, each containing 5 root pieces per sampled seedling, were incubated at about 24°C in the dark for three days. Suspected colonies of *Pythium* and *Phytophthora* were transferred to PDA and water agar for identification using the taxonomy of Middleton (1943), Stamps et al. (1990), and Waterhouse (1956; 1968). Root colonization was expressed as percentage of sampled root pieces colonized by particular fungi.

Water solutions of rhizosphere soil from 5 of the diseased seedlings were blended thoroughly and 1 ml of the solution placed directly onto V-8 juice agar medium. Plates were incubated as described above. The number of *Pythium* and/or *Phytophthora* colonies produced on each plate was determined.

RESULTS AND DISCUSSION

All of the sampled diseased western larch seedlings had roots that were infected with either or both *Phytophthora* and *Pythium* spp. (table 1). Root colonization by *Phytophthora* spp. averaged more than 50%, while *Pythium* spp. colonized slightly fewer roots. Two *Phytophthora* spp. were identified from isolates obtained from diseased seedling roots. The most common was *P. cactorum* (Leb. and Cohn.) Schr.; *P. megasperma* Drech. emend Hamm and Hansen was isolated much less frequently. The three *Pythium* spp. isolated in order of descending intensity were *P. irregulare* Buisman., *P. ultimum* Trow., and *P. aphanidermatum* (Edson) Fitzp. *Phytophthora* and *Pythium* spp. were also commonly isolated from the rhizosphere of diseased seedlings (table 2). More than twice as many colonies of *Phytophthora* were isolated from root rhizospheres than *Pythium*.

Fusarium spp., which have commonly been associated with bareroot seedling root diseases at the Coeur d'Alene Nursery (James 1989c; James et al. 1990), were isolated from 80% of the sampled diseased seedlings (table 3). However, levels of root colonization were generally lower than either *Phytophthora* or *Pythium* spp. The most commonly isolated *Fusarium* spp. was *F. oxysporum* Schlecht.; *F. acuminatum* Ell. & Ev. was isolated less frequently. *Trichoderma* spp., common soil saprophytes and potential antagonists of plant-pathogenic fungi (Papavizas 1985; Papavizas et al. 1980), were isolated from all sampled seedlings, although colonization rates varied widely (table 3).

Based on these isolation results, the most probable causes of western larch seedling mortality, stunting and chlorosis in bareroot beds at the Coeur d'Alene Nursery were *Phytophthora* and *Pythium* spp. *Phytophthora* spp. were probably the more virulent pathogens; these fungi can aggressively attack conifer seedlings in nurseries and induce rapid disease development (Hamm and Hansen 1982, 1985, 1987; Hansen et al. 1980). *Phytophthora* spp. have infrequently been reported at the Coeur d'Alene Nursery; *P. cactorum* was previously found causing basal lesions on western larch tree improvement stock in another field (James 1993). In contrast, *Pythium* spp. are commonly isolated from diseased bareroot seedlings at the nursery (James 1982; James et al. 1991). Both groups of fungi are usually associated with production beds lacking adequate water drainage (James 1982; Hamm and Hansen 1985); both produce water-motile zoospores which facilitate rapid disease spread (Hamm et al. 1984; Middleton 1943; Waterhouse 1956, 1968). Therefore, when soils remain water saturated, disease develops and spreads rapidly. Infected conifer seedlings initially turn chlorotic and may eventually die if their root systems are extensively colonized and decayed by either *Phytophthora* or *Pythium* spp.

(Hansen et al. 1980; James 1982, 1996, 1997).

The most effective way to control *Phytophthora* and *Pythium*-induced root diseases is by pre-plant soil fumigation (James 1989b; James et al. 1990) It is also important to either provide adequate water drainage or grow susceptible seedling crops in fields with proper drainage (Hansen et al. 1980; James 1982, 1997). Application of fungicides as soil drenches usually give variable results after root diseases become evident. It is important that causal organisms are properly diagnosed prior to applying fungicides. If *Phytophthora* and *Pythium* spp. are known to be the most important causes of disease, soil drenching with metalaxyl (Subdue®) may help reduce disease spread and improve survival of infected seedlings (Hamm et al. 1984; Hunger et al. 1982). However, this fungicide is not efficacious against other types of soilborne pathogens, such as *Fusarium* spp.

Although the field in which diseased western larch seedlings were growing had been fumigated with dazomet prior to sowing, apparently sufficient inoculum of *Phytophthora* and *Pythium* survived or reinfected the field to cause seedling infection and disease during the first growing season. Dazomet is applied topically, incorporated into soil with a cultivator, and activated by overhead irrigation (James 1989b; James et al. 1990). Penetration of the fumigant may be limited by soil physical characteristics (particularly porosity) and water content.

In conclusion, it is apparent that both *Phytophthora* and *Pythium* spp. may be important pathogens of bareroot western larch seedlings at the Coeur d'Alene Nursery. These pathogens can either survive in or reinfect fumigated fields in sufficient numbers to cause root disease by the end of the first growing season. Therefore, care must be taken to ensure that adequate water drainage is provided in fields destined to produce western larch seedlings. Although spot treatments with specific water mold fungicides will help, prevention is the best approach to reduce future losses.

Table 1. Colonization of bareroot western larch seedling roots with *Pythium* and *Phytophthora* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedling	Percent Root Colonization[1]	
Number	*Phytophthora* spp.	*Pythium* spp.
1	20	40
2	40	60
3	80	20
4	60	40
5	100	20
6	60	60
7	40	80
8	80	20
9	0	100
10	80	0
Average	56	44

[1] Based on sampling 5 randomly-selected root pieces per seedling.

Table 2. Occurrence of *Phytophthora* and *Pythium* spp. in rhizosphere soil of bareroot western larch seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Sample	Number of Colonies[1]	
Number	*Phytophthora* spp.	*Pythium* spp.
1	0	3
2	8	0
3	8	2
4	3	3
5	5	2
Average	4.8	2.0

[1] Colonies per 1 ml of water/soil solution washed from roots of diseased seedlings.

Table 3. Colonization of bareroot western larch seedling roots with *Fusarium* and *Trichoderma* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedling	Percent Root Colonization[1]			
Number	*F. oxysporum*	*F. acuminatum*	All *Fusarium*	*Trichoderma*
1	0	0	0	60
2	0	0	0	100
3	40	0	40	30
4	40	0	40	50
5	30	30	60	10
6	30	50	80	10
7	20	20	40	30
8	60	10	70	30
9	10	0	10	60
10	30	0	30	40
Average	26	11	37	42

[1] Based on randomly-sampling 10 root pieces per seedling.

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Figure 1. Bareroot western larch seedlings with chlorotic and necrotic foliar symptoms and reduced seedling density indicative of root disease – USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Figure 2. Chlorotic and necrotic bareroot western larch seedlings in low portions of seedbeds where water drainage was impaired – USDA Forest Service Nursery, Coeur d'Alene, Idaho.