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INVESTIGATIONS OF TREE HEALTH AT THE POTLATCH CORPORATION CHERRYLANE SEED ORCHARD, IDAHO

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Abstract

Investigations were conducted from 1998-2000 at the Potlatch Corporation's Cherrylane Seed Orchard near Lewiston, Idaho to evaluate presence and extent of potentially pathogenic fungi on roots of diseased stock and within the soil in current and proposed plantation sites. The most common group of pathogens encountered was *Fusarium* spp.; *F. oxysporum* was by far the most common colonizer of tree roots and soil. *Phytophthora* spp. (*P. cactorum* and *P. pseudotsugae*) and *Pythium* spp. (*P. irregulare* and *P. ultimum*) were also common root and soil colonizers. *Cylindrocarpon* spp. (*C. destructans* and *C. tenue*) were isolated at lower frequencies. Soil solarization with a thin plastic tarp during the summer effectively reduced populations of potential soil-borne pathogens. Managers should continue to monitor pathogen populations on stock and within soil of proposed plantation sites in order to reduce future disease impacts.

Introduction

The Potlatch Corporation's Cherrylane Seed Orchard is located about 10 miles east of Lewiston, Idaho, adjacent to U.S. Highway 12. It contains tree improvement planting stock destined to provide high quality seed for use in production of reforestation seedlings for Potlatch forest lands. Trees established within the seed orchard are of high value and need to be preserved for as long as possible and maintained in healthy conditions.

Managers in charge of the seed orchard have periodically encountered trees with symptoms of insect and disease attacks. In most cases, insect attacks are easily recognized and causal agents identified, so that appropriate prevention and suppression actions can be taken. However, identification of disease-causing agents is usually more difficult and requires laboratory analysis of diseased tree tissues.

A series of diseases have recently been investigated at the seed orchard. One important problem was decline and dieback of western larch (*Larix occidentalis* Nutt.) trees. Roots and adjacent soil of affected trees as well as foliage samples were submitted to a diagnostic laboratory in Washington State in 1997. Isolations from affected roots and soil yielded three groups of potential pathogens: *Phytopthora*, *Fusarium*, *Pythium*, and small amounts of *Cylindrocladium* spp. No evidence of pathogens was found on foliage samples.

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The most common soil-borne pathogen encountered was *Fusarium oxysporum* Schlecht. Based on all samples, personnel at the diagnostic laboratory thought the major cause of larch decline and dieback was root infection by *Phytophthora* spp. They recommended soil drenches with appropriate fungicides, particularly metalaxyl (Subdue®). The following year (1998), roots of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) as well as a second set of root samples from western larch trees with dieback symptoms were submitted to the same laboratory for analysis. High levels of root infection by *Fusarium* and *Cylindrocarpon* spp. were found on Douglas-fir; the western larch samples again yielded *Phytophthora*. Some low amounts of *Rhizoctonia* were also isolated from roots of both conifer species. Laboratory personnel recommended using nitrate nitrogen fertilizer instead of ammonia to help reduce impact of *Fusarium*-associated diseases.

Following these initial diagnoses, more detailed investigations of diseases and associated microorganisms were undertaken from 1998-2000 to help reduce future problems on high-value seed orchard trees; this report summarizes these investigations.

Materials and Methods

All root and soil samples were processed in approximately the same way. Roots (and often adjacent soil) were collected below sample trees, placed in plastic bags, refrigerated, and transported to the laboratory for processing. Passing the samples through a 2 mm sieve separated roots and soil. While fine soil passed through the sieve, root pieces, soil aggregates, and rocks were retained. Roots were washed thoroughly to remove adhering soil particles and cut into pieces approximately 5 mm in length. Root pieces were then surface sterilized in a 10 percent bleach (0.525 percent aqueous sodium hypochlorite) solution for 1 minute, rinsed in sterile water, and blotted dry. They were then placed on an agar medium (KM) selective for *Fusarium* and closely related fungal species (Komada 1975) or for water mold (WM) fungi including *Phytophthora* and *Pythium* spp. (James and Beall 1999; James et al. 1990, 1996). KM plates were incubated for 7-10 days at about 24°C under 12-hour diurnal cycles of cool, fluorescent light. WM plates were incubated at about 24°C for 3 days in the dark. Selected emerging *Fusarium* and water mold fungi were identified using the taxonomy of Nelson et al. (1983), Middleton (1943), Stamps et al. (1990) and Waterhouse (1956, 1968). Generally, root colonization was quantified as the percent of the number of sampled root pieces colonized by particular fungi.

After sieving, soil was refrigerated until processed for populations of potentially pathogenic fungi. In some cases, aggregate soil samples were divided into several individual samples, each processed separately for comparative purposes. From each sample, an approximate 5 g sub sample was oven dried at about 100°C for at least 24 hours, or until sample weight had stabilized. Oven-dry weight was then calculated to provide a standard for sample comparisons. For assay of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3 percent water agar (WA) and thoroughly mixed. One ml of solution was placed on each of three plates of KM and spread uniformly. Plates were incubated as described above. *Fusarium* and *Trichoderma* colonies were identified by their morphology; populations were expressed as number of colony-forming units (CFU) per g of oven-dried soil. Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al., 1982) and potato dextrose agar for identification using the taxonomy of Nelson

and others (1983). For assay of *Pythium* and *Phytophthora* 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 of WM medium. Plates were incubated in the dark at about 24°C for 3 days. *Pythium* colonies were identified based on their diameter after 3 days (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. *Phytophthora* colonies were slower growing and identified by their production of sporangia and/or oospores within the agar medium. For population calculations, it was assumed that each fungal colony originated from one individual propagule.

Table 1. Location and family of origin of large, western larch trees sampled for root and soil pathogens (1999) – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.

Tree Number	Family	Row	Column
1	24309	7	46
2	19908	7	43
3	21305	11	13
4	6104	3	45
5	2110	5	35
6	2110	10	24

The first set of samples was collected from beneath four Douglas-fir trees with evidence of branch dieback. Samples were collected and processed in 1998 and consisted of both soil and root samples from beneath each tree. A second sample was collected in April 1999 from six large western larch trees (Table 1) without apparent disease symptoms; samples were also collected from two healthy-appearing Douglas-fir trees at the same time. These samples consisted of both roots and adjacent soil.

The third set of samples consisted of soil in two locations destined for seedling outplanting. The two sites were designated "east" and "west" based on their location relative to each other. Within the "west" site, located southwest of the office/shop complex, 10 systematic soil samples were collected approximately equidistant from each other in the spring of 1999. Each soil sample consisted of a core taken to a depth of about 15 cm. Soil was placed in labeled plastic bags, kept refrigerated, and processed as described above for populations of *Fusarium*, *Trichoderma*, and water molds (*Pythium* and *Phytophthora* spp.). The ratio of *Trichoderma* to *Fusarium* (T/F) populations was calculated for each sample; this ratio may be useful as an approximation of potential *Fusarium* disease suppressiveness in soils (James and Beall 1999; James et al 1996). Within the "east" site, two sets of soil samples were analyzed. The first consisted of 10 systematic samples collected at the time of the "west" collections. The second sample was taken in the fall of 1999 after the site had been covered with plastic tarp (2 mil) for about 3 months. This post-solarization sample comprised 14 systematic collections. These soil samples were collected and processed as described above.

The fourth sample consisted of root and soil samples from beneath large lodgepole pine trees with "loose" roots, i.e., the stems were easily moved back and forth due to

poor root egress and growth from original planted root plugs (container-grown seedlings). Root and soil samples from four trees were combined into one large sample. Roots were processed for pathogen colonization as described above; soil was divided into 10 samples and processed as described above.

The fifth and final sample consisted of five container-grown western larch seedlings that had been used as rootstock grafting material. The seedlings were either dead or near death; some seedlings had evidence of substantial root decay, whereas others had very little decay. Roots of sampled seedlings were washed thoroughly under running tap water for several minutes to remove as much growth medium and soil as possible. The root systems were processed as described above and pieces incubated on KM and WM media. Percentage of root systems colonized by specific potentially pathogenic fungi was calculated.

Results

Isolation results from roots of large Douglas-fir trees with branch dieback symptoms are summarized in Table 2. About two-thirds of the sampled roots were colonized with *Fusarium* spp. The most commonly isolated *Fusarium* species was *F. oxysporum*, obtained from slightly more than 50 percent of the root samples tested. Another common *Fusarium* isolated from tree roots was *F. equiseti* (Corda) Sacc. *Fusarium* soil populations were quite variable beneath the sampled Douglas-fir trees (Table 3); relatively high populations were found beneath trees 2 and 4. The major soil-inhabiting *Fusarium* species was *F. oxysporum*; other isolated species included *F. culmorum* (W.G. Smith) Sacc., *F. acuminatum* Ell. & Ev., and *F. solani* (Mart.) Appel & Wollenw. Soil populations of *Trichoderma* were also quite variable (Table 3). These organisms were frequently isolated on KM; some species of *Trichoderma* may be suppressive to *Fusarium* pathogens (Papavizas 1985; Papavizas and Lumsden 1980); the higher the ratio of *Trichoderma* to *Fusarium* populations, the more potentially suppressive the soil is to *Fusarium*-elicited diseases (James and Beall 1999; James et al. 1996). Water mold fungi (*Pythium irregulare* Buisman, *P. ultimum* Trow and *Phytophthora pseudotsugae* Hamm and Hansen) commonly colonized the soil beneath sampled Douglas-fir trees (Table 3). However, their populations were generally below levels considered potentially damaging to conifers (Hildebrand and Dinkel 1988; James and Beall 1999).

Table 2. *Fusarium* colonization of roots of Douglas-fir trees with branch dieback symptoms – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.

	Fusarium Colonization [1]		
Tree Number	*F. oxysporum*	*F. equiseti*	All *Fusarium*
1	35	0	35
2	50	40	85
3	50	10	60
4	80	10	85
Average	53.8	15.0	66.3

[1] Percent of root pieces (20 sampled per tree) colonized by appropriate *Fusarium* sp.

Table 3. Soil populations of selected fungi from samples beneath Douglas-fir trees with branch dieback symptoms – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Sample No.	*Fusarium*[2]	*Trichoderma*	T/F Ratio[3]	Water Molds[4]
1	337	4369	13.0	54
2	1416	674	0.5	100
3	135	135	1.0	41
4	1548	337	0.2	81
Average	859	1378	1.6	69

[1] Numbers are colony-forming units per g oven-dried soil.
[2] Percent of isolates: *F. oxysporum* = 92.1%; *F. culmorum* = 3.9%; *F. acuminatum* = 2.0%; *F. solani* = 2.0%.
[3] Ratio of *Trichoderma* to *Fusarium* populations.
[4] Includes both *Phytophthora* (*P. pseudotsugae*) and *Pythium* (*P. irregulare* & *P. ultimum*) spp.

Isolation results from the 1999 collections of roots of western larch and Douglas-fir trees are summarized in Table 4. *Fusarium* spp. were isolated from the roots of 83 percent of the sampled western larch trees, although rates of root colonization were quite low. *Fusarium* spp. were found on the roots of both sampled Douglas-fir trees, at higher colonization rates than on western larch trees. Most of the *Fusarium* isolates obtained from roots were *F. oxysporum; F. solani* was also frequently isolated. *Phytophthora cactorum* (Leb. and Cohn.) Schr. and *P. pseudotsugae* were isolated from four sampled larch and one sampled Douglas-fir. Rates of *Phytophthora* root colonization were very low. *Cylindrocarpon* spp. were also frequently isolated from western larch and Douglas-fir roots; these root-colonizing fungi, which may occasionally be pathogenic (James et al. 1994), were commonly isolated from roots of all sampled western larch and one of the sampled Douglas-fir. Rates of *Cylindrocarpon* root colonization on western larch were higher than for *Fusarium* and *Phytophthora* spp. The two major *Cylindrocarpon* species isolated from roots were *C. destructans* (Zins.) Scholten and *C. tenue* Bugn. Saprophytic *Trichoderma* spp. were also frequently isolated from roots of all sampled western larch and Douglas-fir trees.

Table 4. Colonization of roots of large western larch and Douglas-fir trees by selected fungi – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Tree Number[2]	*Fusarium*[3]	*Phytophthora*[4]	*Cylindrocarpon*[5]	*Trichoderma*
Western Larch				
1	10.0	0	31.7	31.7
2	35.0	0	41.7	21.7
3	6.7	6.7	8.3	43.3
4	13.3	6.7	43.3	35.0
5	0	20.0	13.3	71.7
6	15.0	33.3	25.0	45.0
Average	13.3	11.1	27.2	41.4
Douglas-fir				
1	25.0	0	0	83.3
2	43.0	13.3	33.3	10.0
Average	34.2	6.7	16.7	46.7

[1] Numbers are percent of root pieces colonized by appropriate fungus (60 sampled/tree for *Fusarium*, *Cylindrocarpon* and *Trichoderma* assays; 15 sampled/tree for *Phytophthora* assays).
[2] See Table 1 for location and origin of sampled western larch trees; western larch trees were mostly without above-ground disease symptoms; Douglas-fir trees were fading with chlorotic and necrotic portions of crowns.
[3] Percent of isolates: *F. oxysporum* = 70.2 percent; *F. solani* = 29.8 percent.
[4] Primarily *P. cactorum* (western larch) and *P. pseudotsugae* (Douglas-fir).
[5] Primarily *C. destructans* and *C. tenue*.

Soil sampled beneath those western larch and Douglas-fir trees whose roots were analyzed for pathogenic fungi (Table 4) yielded low to moderate populations of *Fusarium*, relatively high populations of *Trichoderma*, and very low populations of *Phytopthora* and *Pythium* spp. (Table 5). *Fusarium* spp. colonizing soil included *F. oxysporum*, *F. equiseti*, *F. solani* and F. *sporotrichioides* Sherb.; *F. oxysporum* was by far the most common soil-inhabiting *Fusarium* sp. *Trichoderma* populations were substantially higher than *Fusarium* populations, particularly under western larch trees. The high *Trichoderma* to *Fusarium* ratios may indicate disease suppressiveness of soil. Soil populations of water mold fungi were made up of *Phytophthora cactorum* and *Pythium irregulare* and *P. ultimum*.

Soil *Fusarium* populations in the western field destined for plantation establishment were very high (Table 6). *Fusarium* populations for the ten samples averaged more than 10,000 cfu/g of soil, many orders of magnitude higher than accompanying *Trichoderma* populations. *Trichoderma/Fusarium* ratios for the entire site were very low, indicating that the soil might be highly conducive to *Fusarium* disease if susceptible crops are planted. Such high *Fusarium* populations, even without associated low *Trichoderma* levels, would be expected to elicit severe disease (Hildebrand and Dinkel 1988; James and Beall 1999). The vast majority of the *Fusarium* population consisted of *F. oxysporum; F. solani* and *F. equiseti* were isolated at very low levels. *Phytopthora* and *Pythium* populations within this western plantation site were quite variable. Some samples yielded relatively high populations (> 100 cfu/g), indicating some disease potential (Hildebrand and Dinkel 1988).

Table 5. Soil populations of selected fungi beneath large western larch and Douglas-fir trees – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Tree Number[2]	*Fusarium*[3]	*Trichoderma*	T/F Ratio[4]	Water Molds[5]
Western Larch				
1	212	1380	6.5	0
2	637	2682	4.2	0
3	168	1007	6.0	0
4	34	1106	32.5	7
5	100	6063	60.6	0
6	402	1942	4.8	17
Average	259	2363	9.1	4
Douglas-fir				
1	201	2073	10.3	20
2	1303	267	0.2	47
Average	752	1170	1.6	34

[1] Numbers are colony-forming units per g oven-dried soil.
[2] See Table 1 for location and origin of sampled western larch trees; western larch trees were mostly without above-ground disease symptoms; Douglas-fir trees were fading with chlorotic and necrotic portions of crowns.
[3] Percent of isolates: *F. oxysporum* = 87.4 percent; *F. equiseti* = 9.2 percent; *F. solani* = 1.1 percent; *F. sporotrichioides* = 2.3 percent.
[4] Ratio of *Trichoderma* to *Fusarium* populations.
[5] Includes both *Phytophthora* (*P. cactorum*) and *Pythium* (*P. irregulare* & *P. ultimum*) spp.

Table 6. Pre-plant soil populations of *Fusarium*, *Trichoderma*, *Pythium* and *Phytophthora* spp. in a western plantation site – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Sample No.	*Fusarium*[2]	*Trichoderma*	T/F Ratio[3]	Water Molds[4]
1	6384	269	0.04	47
2	10213	269	0.03	7
3	10014	134	0.01	27
4	6524	336	0.05	34
5	13971	135	0.01	40
6	12293	338	0.03	108
7	7826	607	0.08	128
8	12707	471	0.04	128
9	13282	202	0.01	142
10	9485	605	0.06	208
Average	10270	337	0.03	87
STD[5]	2619	165	--	62

[1] Site located southwest of office/shop complex; numbers are colony-forming units per g oven-dried soil.
[2] Percent of isolates: *F. oxysporum* = 99.4 percent; *F. solani* = 0.5 percent; *F. equiseti* = 0.1 percent.
[3] Ratio of *Trichoderma* to *Fusarium* populations.
[4] Includes both *Phytophthora* and *Pythium* spp.
[5] Standard deviation.

Background *Fusarium* population levels were much lower in the eastern plantation site, averaging just over 1000 cfu/g (Table 7). Solarization for 3 months with a thin plastic tarp had dramatic effects on soil *Fusarium* populations (Table 7) but less effects on *Phytophthora* and *Pythium* populations (Table 8) at this site. Solar heating decreased *Fusarium* populations at most sample locations with average populations being reduced by about half. As with previous soil analyses, *F. oxysporum* was by far the most common *Fusarium* species isolated from soil before and after solarization. Average *Trichoderma* populations were not significantly affected by solarization (Table 7). *Trichoderma* to *Fusarium* ratios greatly increased following solarization, indicating that treated soil might be more disease suppressive than non-solarized soil. Water mold fungi were at relatively low levels both before and after solarization treatments (Table 8). Major assayed water mold organisms included *Phytophthora cactorum*, *Pythium irregulare* and *P. ultimum.*

Table 7. Effects of solarization on pre-plant soil populations of *Fusarium* and *Trichoderma* spp. in an eastern plantation site – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Sample	Pre-Solarization			Post-Solarization		
Number	FUS[2]	TRI[3]	T/R[4]	FUS[5]	TRI[3]	T/R[4]
1	738	1410	1.9	1138	1405	1.2
2	1074	2753	2.6	669	2409	3.6
3	536	2882	5.4	334	0	0
4	1608	603	0.4	67	6348	94.8
5	1277	1478	1.2	267	2941	11.0
6	738	1410	1.9	802	1069	1.3
7	604	402	0.7	267	1270	4.7
8	1008	1612	1.6	1069	6348	5.9
9	1476	5771	3.9	67	2338	34.9
10	1276	873	0.7	735	0	0
11	--	--	--	134	67	0.5
12	--	--	--	601	2538	402
13	--	--	--	1136	200	0.2
14	--	--	--	668	936	1.4
Average	1034	1919	1.8	568	1991	11.7
STD[6]	354	1496	--	371	2020	--

[1] Numbers are colony-forming units per g oven-dried soil.
[2] *Fusarium* spp.; percent of isolates: *F. oxysporum* = 72.0 percent; *F. equiseti* = 22.3 percent; *F. sporotrichioides* = 5.1 percent; *F. solani* = 0.6 percent.
[3] *Trichoderma* spp.
[4] Ratio of *Trichoderma* to *Fusarium* populations.
[5] *Fusarium* spp.; percent of isolates: *F. oxysporum* = 97.5 percent; *F. equiseti* = 1.7 percent; *F. solani* = 0.8 percent.
[6] Standard deviation.

Table 8. Effects of solarization on pre-plant soil populations of Phytophthora and Pythium spp. in an eastern plantation site – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Sample Number	Pre-Solarization	Post-Solarization
1	47	74
2	34	13
3	27	0
4	7	0
5	40	7
6	54	33
7	40	20
8	20	20
9	54	7
10	13	13
11	--	0
12	--	7
13	--	7
14	--	0
Average	34	19
Standard Deviation	16	19

[1] Numbers are colony-forming units of *Phytophthora* and *Pythium* spp. per g oven-dried soil; *Phytophthora* spp. comprised mostly of *P.* cactorum; *Pythium* spp. includes *P. irregulare* and *P. ultimum.*

Root colonization analyses of large lodgepole pine trees with "loose" roots are summarized in Table 9. *Fusarium* spp. were isolated from half of the sampled root pieces; *F. oxysporum* was again the most common species encountered. *Pythium irregulare* was common on sampled root pieces as well. Other potentially pathogenic fungi isolated from sampled roots included *Cylindrocarpon destructans* and *Phytophthora cactorum*. *Fusarium* populations in soil adjacent to these lodgepole pine trees, although quite variable, averaged low levels (Table 10). *Trichoderma* populations in these soils were much higher, resulting in relatively high average *Trichoderma* to *Fusarium* ratios. Water mold fungi were found at very low levels within these soils.

Table 9. Colonization of roots of large lodgepole pine trees with "loose" root systems by selected fungi – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.

Fungus	Percent Root Colonization[1]
Fusarium oxysporum	43.5
Fusarium solani	6.5
All *Fusarium*	50.0
Cylindrocarpon destructans	13.0
Pythium irregulare	38.7
Phytophthora cactorum	1.3
Trichoderma spp.	2.8
Penicillium spp.	31.5

[1] Based on sampling 108 root pieces (~5 mm length) for *Fusarium, Cylindrocarpon, Trichoderma* and *Penicillium* and 75 root pieces for *Pythium* and *Phytophthora* spp.

Table 10. Soil populations of *Fusarium, Trichoderma, Pythium* and *Phytophthora* spp. under large lodgepole pine trees with "loose" roots – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Sample No.	*Fusarium*[2]	*Trichoderma*	T/F Ratio[3]	Water Molds[4]
1	67	1277	19.1	13
2	470	874	1.9	0
3	67	806	12.0	34
4	403	1277	3.2	13
5	134	672	5.0	7
6	806	538	0.7	7
7	269	672	2.5	13
8	336	874	2.6	20
9	134	605	4.5	27
10	336	336	1.0	7
Average	302	793	2.6	14
STD[5]	227	302	--	10

[1] Numbers are colony-forming units per g oven-dried soil.
[2] Percent of isolates: *F. oxysporum* = 86.7 percent; *F. equiseti* = 8.9 percent; *F. solani* = 4.4 percent.
[3] Ratio of *Trichoderma* to *Fusarium* populations
[4] Includes both *Phytophthora* and *Pythium* species.
[5] Standard deviation.

Roots of grafted container-grown western larch seedlings were extensively colonized by *Fusarium* spp. (Table 11). Four different *Fusarium* spp. were isolated from seedling roots: *Fusarium acuminatum* was the most common, followed by *F. sporotrichioides* and *F. proliferatum* (Matsushima) Nirenberg and *F. culmorum*. *Phytophthora* spp. were isolated at very low levels from the roots of two of five sampled seedlings (Table 11).

Table 11. Colonization of container-grown western larch grafted seedlings with *Fusarium, Trichoderma* and *Phytophthora* spp. – Potlatch Corporation, Cherrylane Seed Orchard, Idaho.[1]

Seedling	*Fusarium* Colonization[2]					Other Fungi[3]	
Number	FPRO	FACU	FSPO	FCUL	All FUS	TRI	PHYTO
1	35.0	0	0	5.0	40.0	20.0	0
2	5.0	0	90.0	0	95.0	0	0
3	0	85.0	0	0	85.0	0	10
4	5.0	90.0	0	0	95.0	0	10
5	5.0	90.0	0	0	95.0	10.0	0
Average	10.0	53.0	18.0	1.0	82.0	8.0	4.0

[1] Based on sampling 20 root pieces per seedling for *Fusarium* and *Trichoderma* and 10 roots per seedling for *Phytophthora*; numbers are percent of sampled pieces colonized with appropriate fungus.
[2] FPRO = *F. proliferatum*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides*; FCUL = *F. culmorum*.
[3] TRI = *Trichoderma* spp.; PHYTO = *Phytophthora* spp.

Discussion

The Potlatch Cherrylane Seed Orchard site is on agricultural land previously used for livestock pasture. The soil mycoflora consists of microorganisms that may be potential pathogens of conifer seedlings and trees, particularly when these plants are produced under the intensive management schemes common in tree improvement plantations. The pathogens encountered in these investigations can also potentially colonize conifer seedling stock being produced in nurseries. Experience has indicated that pathogens are sometimes carried on roots of stock from nurseries (James 1991, 1995). However, because many of the same organisms are also residents of plantation sites, it is difficult to determine proportions of these organisms which may have been brought in on nursery stock.

Many of the fungi commonly isolated from trees and soil in these investigations may or may not be pathogenic under differing environmental conditions. For example, some strains of *Fusarium* are pathogens while others are saprophytes; often both pathogenic and saprophytic strains are morphologically identical. The most common way to differentiate pathogenic from non-pathogenic *Fusarium* strains is to test representative isolates for their ability to induce diseases under carefully controlled conditions. These pathogenicity tests are tedious, time-consuming, and expensive. Other approaches, including genetic differentiation of strains, provide feasible means of quickly separating strains into pathogens and nonpathogens (Appel and Gordon 1995; Gordon and Martyn 1997; Gordon and Okamoto 1992). Such techniques have not been refined for all the *Fusarium* spp. commonly obtained at the Cherrylane Seed Orchard, but it is likely that such techniques will become readily available in the future.

Because of the common presence of potentially pathogenic organisms on roots and surrounding soil of both healthy and non-healthy trees, cause and effect relationships between the organisms and tree disease symptoms is often hard to establish. Because some fungi capable of eliciting disease are isolated from the roots of trees with branch dieback symptoms, this does not necessarily mean these organisms caused the symptoms. Environmental factors such as temperature, soil moisture, and nutrition, may be as or more important than presence of certain microorganisms. Many fungi isolated from roots of apparently diseased trees become pathogenic only when trees are sufficiently stressed (James et al. 1991). When tree stresses are of sufficient magnitude, even relatively low pathogen populations may be capable of causing diseases. On the other hand, if trees are not sufficiently stressed, even large populations of potential pathogens may be incapable of causing disease.

One definitive conclusion of this evaluation was that background soil populations of *Fusarium* spp. were of sufficient numbers to be of concern when transplanting seedling stock into particular fields. *Fusarium* soil populations in excess of about 1000 cfu/g are usually considered high enough to elicit some level of disease in susceptible conifer seedlings (Hildebrand and Dinkel 1988; James and Beall 1999). As populations increase beyond this threshold, disease potential increases. Several soil samples from different locations within the seed orchard exceeded this threshold, especially those in the proposed "western" plantation site. Subjecting soil to solarization treatment by covering with a thin transparent plastic tarp during the summer was sufficient to greatly reduce soil *Fusarium* levels. This technique may have widespread efficacy at the site because of the relatively porous soils and characteristic high summer temperatures. Solarization treatments may preclude the need for chemical pesticide treatments to reduce soil pathogen levels.

The major *Fusarium* pathogen of concern at the seed orchard is *F. oxysporum*. This species is often the most important soil-borne pathogen in many agricultural sites as well as

nurseries producing bareroot conifer seedlings (Bloomberg 1971; Gordon and Martyn 1997; James et al. 1991; Matuo and Chiba 1966). *Fusarium oxysporum* is a broadly defined morphological species that is made up of many genetically differentiated strains (Gordon and Martyn 1997; Kistler 1997). Strains pathogenic to specific plant hosts are defined as formae speciales, or form species capable of eliciting disease only on particular hosts (Armstrong and Armstrong 1975; Matuo and Chiba 1966). In addition, there is a wide diversity of saprophytic strains of *F. oxysporum*, some of which may be antagonistic toward pathogenic strains (Alabouvette et al. 1993; Lemanceau and Alabouvette 1991). Both pathogenic and non-pathogenic strains appear morphologically similar, although they may have important genetic differences (Gordon and Martyn 1997; Kistler 1997). Investigation techniques at the Cherrylane Seed Orchard did not differentiate among strains of *F. oxysporum*. Some proportion of the soil population was undoubtedly made up of pathogenic strains. However, quantitative estimates of this proportion were not made. Based on previous experience (Hildebrand and Dinkel 1988; James and Beall 1999; James et al. 1996), when soil populations reach or exceed certain threshold levels (see above), some level of disease should be expected.

Another important group of potential pathogens frequently encountered in these investigations was *Phytophthora*. These important soil-borne fungi may be extremely aggressive pathogens (Hamm et al. 1984; Waterhouse 1956) and cause root deterioration and decay on a wide variety of plants. Most conifer species are susceptible to *Phytophthora* spp. (Hamm and Hansen 1982, 1987). In the inland Pacific Northwest, Douglas-fir (Hamm and Hansen 1983; James 1997) and western larch (James 1993, 2000) seem particularly susceptible. These important pathogens can cause extensive plant damage, even at relatively low populations. When soil populations of *Pythium* and/or *Phytophthora* exceed about 100 cfu/g, some level of disease can be expected on susceptible host plants (Hildebrand and Dinkel 1998; James and Beall 1999). Unlike *Fusarium* spp., a greater proportion of *Phytophthora* isolates from soil and plant roots are pathogens (Hamm and Hansen 1982, 1985; Hansen et al. 1980). Therefore, when *Phytophthora* spp. are detected, they are usually of more concern from a disease management standpoint.

These investigations helped elucidate presence, level, and identity of potentially pathogenic fungi on seedling and tree roots and the soil at the Cherrylane Seed Orchard. Several of these organisms undoubtedly play an important role in disease initiation on conifer stock. Their numbers within the soil can be reduced by solarization and by spot treatment with fungicide drenches. Even though definitive cause-and-effect relationships between isolated organisms and diseases were not determined, it is recommended that seed orchard managers take steps to maintain low populations of resident pathogens. First periodically monitoring soil populations, particularly in fields destined for new plantations, may accomplish this. If populations of potential pathogens exceed disease threshold levels, some sort of ameliorating treatment is recommended. Another important preventative strategy is to ensure that incoming stock from nurseries is essentially pathogen free. This will require close examination of above- and below-ground portions of stock and subjecting questionable material to laboratory analysis for presence of pathogens. Disease prevention will be much more efficacious than trying to reduce impacts once diseases have occurred.

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