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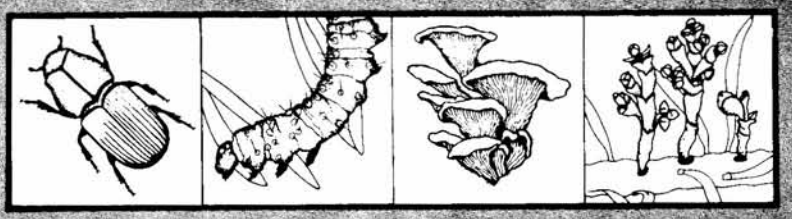

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PHYTOPHTHORA ROOT CROWN DISEASE OF WESTERN LARCH AT THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Foliar chlorosis and mortality of western larch seedlings within a tree improvement plantation at the USDA Forest Service Nursery, Coeur d'Alene, Idaho was caused by basal cankers resulting from infection by *Phytophthora cactorum*. This fungus caused necrosis of phloem, cambium, and outer xylem tissues near the groundline. Cankers extended a few mm below groundline acropetally up to 50 mm. The pathogen was not detected in roots of affected seedlings; low levels of *Phytophthora* were detected in soil adjacent to diseased seedlings. This is the first report of *Phytophthora* at the nursery and also the first report of *P. cactorum* naturally occurring on western larch.

INTRODUCTION

Western larch (*Larix occidentalis* Nutt.) is an important commerical forest tree species in the inland Rocky Mountains. This species is highly prized for its growth rate and high-quality wood. In recent years, desirability of western larch as an important component of forest stands has escalated because it is not often damaged by natural root pathogens, especially when compared with more susceptible species such as Douglas-fir and grand fir (Hadfield and Johnson 1976).

Because of its desirability, an aggressive tree improvement program was implemented for western larch in the Northern Region of the USDA Forest Service. As a portion of this program, early selection trial plantations are periodically established to quantify early performance of selected genotypes. One such early selection trial was established in 1987 in the southwest corner of the USDA Forest Service Nursery in Coeur d'Alene, Idaho. The plantation consisted of container-grown seedlings from selected families spaced at predetermined intervals. Seedling replications within the plantation were randomly placed.

During the middle of the second growing season following outplanting, several trees within the plantation began to display decline symptoms. Affected trees were extensively chlorotic when compared with nearby unaffected trees (figure 1). The entire crown was often equally affected. Affected trees appeared to be growing at comparable rates as unaffected trees and were located throughout the plantation. In some cases two or more diseased trees were located next to each other, but in others such trees were isolated and surrounded by healthy-appearing trees. Careful examination indicated that some trees adjacent to declining ones had died the previous growing season.

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Because of the importance of this plantation and the need to document losses for use in tree improvement evaluations, an investigation was conducted to elucidate the cause of this western larch decline and death.

Figure 1--Diseased western larch tree with pre-mature chlorotic foliage (June) within the tree improvement plantation, USDA Forest Service Nursery, Coeur d'Alene.

MATERIALS AND METHODS

Ten diseased trees with chlorotic foliage were selected during the summer of 1989 for analysis. Trees were removed from the plantation with care so that most of the root system was obtained. Selected trees were located from throughout the plantation and were not concentrated in any particular area. Trees were examined carefully for tissue necrosis within root systems as well as on the main stem. Chlorotic needles were examined microscopically for presence of *Meria laricis* Vuill. because this foliar pathogen commonly occurs on western larch at the nursery (James 1985). Needles were stained with cotton blue and examined for presence of sporodochia emerging from stomata.

Roots were washed thoroughly to remove soil and divided into two categories: "plug" roots produced during the container production phase and "egressed" roots grown from the plug since outplanting. Roots from each category were cut into pieces approximately 2-3 cm in length, surface sterilized in a 10 percent bleach solution (0.525 percent aqueous sodium hypochlorite) for 1 minute and rinsed with sterile water. From each tree, ten randomly selected pieces from the plug and egressed categories were placed on two selective agar media. One medium was selective for *Fusarium* spp. and closely related fungi (Komada 1975), whereas the other was

selective for water mold fungi (*Pythium* and *Phytophthora*). This latter medium was composed of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene. Roots on Komada's medium were incubated under diurnal cycles of fluorescent light at about 24°C for 7-10 days. Roots on V-8 juice agar were incubated at about 22°C for 3 days in the dark. Isolates obtained from roots were placed into different groups based on gross morphology on Komada's medium; representative isolates from each group were transferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher and others 1982) to aid in identification. Fungi growing rapidly over the surface of V-8 juice agar were quantified and representative examples transferred to PDA for identification. Several taxonomic guides (Booth 1966; Middleton 1943; Nelson and others 1983; Waterhouse 1963, 1968) were used to identify the associated isolates.

Since each sampled seedling had a distinct basal canker (see Results and Discussion), the canker size was measured and isolations made from necrotic tissues on canker margins to determine associated fungi. Percent circumference girdled by cankers was estimated. Representative pieces of necrotic phloem and cambial tissue on the margin of each canker were aseptically excised, surface sterilized, and incubated on the selective agar media described above. Number of pieces sampled per seedling varied with canker size.

Soil was collected from five locations adjacent to diseased seedlings for assay of potential pathogenic fungi. Collections were taken to a depth of about 15 cm with a circular soil probe (core diameter = 23 mm). Each sample consisted of four cores collected about 20 cm from the base of each selected seedling and mixed together. Soil was initially sieved to remove large rocks, pieces of organic matter, and soil aggregations. From each soil sample, a 5g subsample was used to calculate oven-dry weight, which provided a standard basis for comparison. For this determination, samples were dried at about 100°C for at least 24 hours or until sample weight had stabilized (all excess moisture removed). For analysis of potential pathogen populations, field-moist soil was used, but fungal populations were reported on an oven-dry weight basis. Four groups of fungi (*Fusarium*, *Pythium*, *Phytophthora*, and *Trichoderma*) were assayed from each soil sample. For assay of *Fusarium* and *Trichoderma* populations, 0.5g of soil was weighed from each sample, combined with 100 ml of 0.3 percent water agar (WA) and thoroughly mixed. One ml of solution was placed on each of three plates of Komada's medium and spread uniformly over the agar surface with a sterile glass rod. Plates were incubated as described previously. *Fusarium* and *Trichoderma* colonies were determined by their morphology on this medium and colony-forming units per g (cfu/g) of soil calculated. Similar procedures were used for assay of *Pythium* and *Phytophthora* populations, except 5 g of soil was initially introduced into WA and the solution placed on the V-8 juice selective medium and incubated as described previously. After incubation, excess soil was carefully washed from the surface of plates and number of *Pythium* and *Phytophthora* colonies determined. *Pythium* colonies were readily determined on the basis of their rapid and diffuse growth habit within and on the medium surface. *Phytophthora* isolates were slower growing and had a more compact growth habit. Suspected isolates were transferred to cornmeal agar containing pimaricin (Hamm and Hansen 1983) for identification. Cfu/g of soil were also calcuated for *Pythium* and *Phytophthora*.

RESULTS AND DISCUSSION

All seedlings with chlorotic foliage and decline symptoms had prominent basal cankers located near the groundline (figure 2) and none were infected with *M. laricis*. Cankers extended up to about 50 mm above the groundline and encircled affected trees, killing phloem, cambial, and outer xylem tissues. Cankers did not extend into the root systems, but were confined to the main stem. Cankered tissue was easily discernible by its darkened color and impregnation with resin (figure 2). In most cases, examined diseased seedlings had healthy-appearing root systems. There was limited root decay in some trees, but such decay was restricted mostly to plug roots and was uncommon on egressed roots.

Figure 2--Basal canker on western larch tree in its second growing season within the tree improvement plantation, USDA Forest Service Nursery, Coeur d'Alene.

Root isolations indicated extensive colonization of both plug and egressed roots with *Cylindrocarpon destructans* (Zins.) Scholten (table 1). Roots of all sampled seedlings were colonized with this fungus; it was isolated at slightly higher levels on egressed roots, but colonization was not associated with noticeable root decay. Although capable of causing root diseases (James 1991; Unestam and Beyer-Ericson 1990), *C. destructans* is a very common colonizer of the rhizosphere of many plant species, often without causing disease symptoms (Evans and others 1967; Matturi and Stenton 1964). Three species of *Fusarium* were also isolated from roots. *Fusarium proliferatum* (Matsushima) Nirenberg was located only on plug roots produced during the container production phase. *Fusarium oxysporum* Schlecht. was isolated most frequently from egressed roots, indicating that infection likely occurred from inoculum residing in soil after outplanting. The other *Fusarium* species isolated from roots was *F. acuminatum* Ell. & Ev. This species was found at very low levels on both plug and egressed roots. All total, *Fusarium* spp. were isolated from most diseased seedlings, but root colonization percentages were quite low and occurrence of these organisms was not associated with noticeable root necrosis or decay. The only water mold fungi isolated from roots were two species of *Pythium*, *P. ultimum* Trow and *P. aphanidermatum* (Edson) Fitzpatrick. Both fungi occurred at low levels on seedling roots, although most seedlings had some infection.

Isolations from basal canker tissue consistently yielded *Phytophthora cactorum* (Leb, & Cohn) Schroet. (table 2). This fungus was readily isolated from surface-sterilized necrotic tissues from canker margins. Most isolation attempts yielded this fungus, although in one case (seedling 4 - table 2), *P. cactorum* was not isolated. *Phytophthora cactorum* falls within taxonomic group I of the genus as described by Waterhouse (1963). The species is delimited primarily on the basis of distinctly papillate sporangia (figure 3) which are produced abundantly on solid substrates, usually deciduous and not proliferating internally. The species also always forms oospores in the host and in culture (homothallic) and antheridia are nearly all paragynous (Waterhouse 1963).

Soil populations of selected fungi are summarized in table 3. *Fusarium* populations were quite low and detected in only two of five samples. *Pythium* and *Phytophthora* spp. were also detected at relatively low levels, although some *Phythophthora* was assayed in four of five samples. It should be noted that populations of *Fusarium* and water mold fungi such as *Pythium* and *Phytophthora* are not directly comparable, at least on the basis of disease-producing potential (Mitchell 1978). Generally, it takes about 10 times as much *Fusarium* inoculum to cause comparable disease as water mold inoculum, i.e., 1000 cfu/g of *Fusarium* would be comparable to 100 cfu/g of water mold fungi. *Trichoderma* spp., which may be anatagonistic toward pathogens such as *Fusarium*, *Pythium*, and *Phytophthora* (Papavizas 1985), were obtained frequently in soil dilution analyses (table 3).

Table 1--Root colonization of diseased western larch within the tree improvement plantation at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1]

	Plug Roots		Egressed Roots[3]	
Isolated[2] Fungus	Seedlings Infected (%)	Root[4] Colonization (%)	Seedlings Infected (%)	Root[4] Colonization (%)
Cylindrocarpon destructans	100	64	100	75
Fusarium proliferatum	90	30	0	0
Fusarium oxysporum	10	2	80	14
Fusarium acuminatum	10	1	10	4
All *Fusarium*	90	33	80	18
Pythium ultimum	30	3	80	14
Pythium aphanidermatum	0	0	30	4
All *Pythium*	30	3	80	18

[1] Trees were plug + 2 (container seedlings outplanted in the tree improvement plantation for two growing seasons). Ten diseased trees were sampled.

[2] *Cylindrocarpon* and *Fusarium* spp. obtained from root isolates on Komada's medium (Komada 1975); *Pythium* spp. from isolations on V-8 juice agar amended with antibiotics and fungicide.

[3] Roots grown from original plug produced during container operations.

[4] Percent of roots sampled (10 per seedling) colonized with appropriate fungus.

Table 2--Frequency of isolation of *Phytophthora cactorum* from root crown tissue of diseased western larch within the tree improvement plantation at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Seedling[1] Number	Basal Canker[2] Percent	Basal Canker[2] Extension	Percent Colonization[3]
1	50	50	83
2	50	30	100
3	70	40	100
4	30	20	0
5	80	40	100
6	40	20	83
7	50	30	80
8	30	30	100
9	40	30	100
10	30	40	71
Avg.	47	33	84

[1] Corresponds to seedlings assayed for root infection.

[2] Characteristics of basal cankers:
percent - percentage of circumference of seedling that has been girdled by canker.
extension - distance (mm) that canker extends from the groundline up the stem.

[3] Based on percent of sampled tissue pieces excised from canker margins that were colonized with *P. cactorum*.

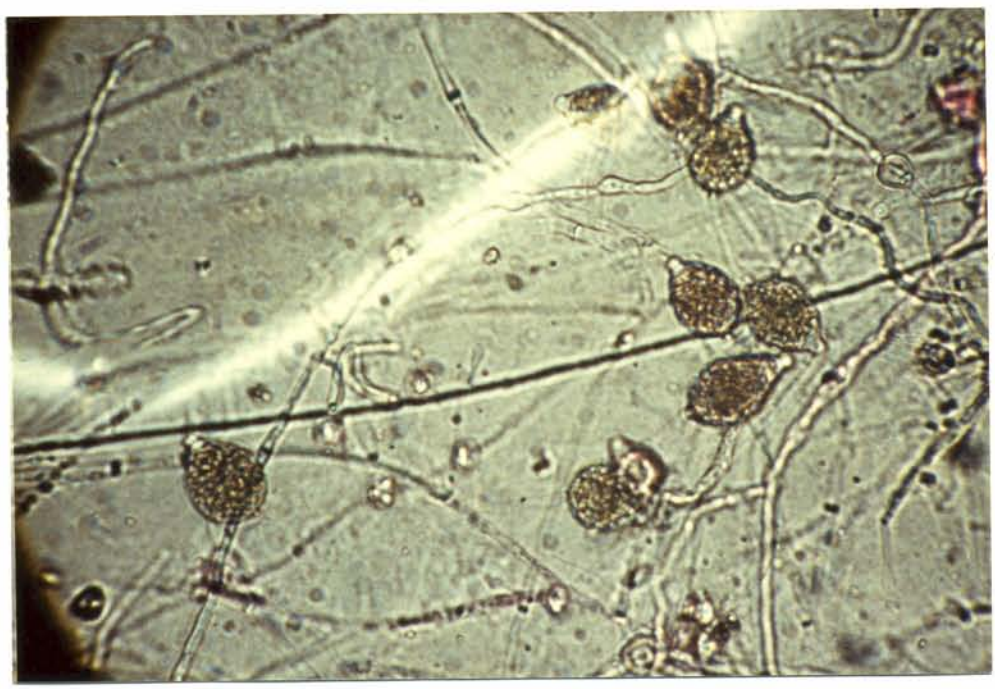

Figure 3--Distinctly papillate sporangia of *Phytophthora cactorum* isolated from basal cankers of western larch seedlings within the tree improvement plantation, USDA Forest Service Nursery, Coeur d'Alene.

Table 3--Soil populations of selected fungi within the western larch tree improvement plantation at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

	Population (cfu/g)[2]			
Sample Number	*Fusarium*	*Pythium*	*Phytophthora*	*Trichoderma*
1	133	53	13	200
2	0	0	40	200
3	0	0	0	0
4	133	40	40	67
5	0	13	20	67
Avg.	53	21	23	107

[1] Based on soil dilution assay on selective agar media.

[2] Based on oven-dry weight of soil.

This evaluation revealed that the primary cause of decline and mortality of western larch within the tree improvement plantation was canker formation caused by *P. cactorum*. This is the first confirmed report of *Phytophthora* at the Coeur d'Alene Nursery. This fungus was consistently isolated from basal cankers, but was not found on roots of any diseased trees sampled. Apparently, the fungus attacked directly at the groundline rather than through the roots. Cankers extended around the circumference of seedlings and upward to a limited degree along the main stem, but did not extend more than a few mm below the groundline. The decline symptom of chlorotic foliage was primarily due to the fungus killing phloem, cambial and outer xylem tissues, essentially girdling the stem. Rate of girdling was not ascertained, but it was probably rather rapid since growth rates of affected seedlings were not greatly reduced prior to onset of disease symptoms or seedling death.

Phytophthora spp. are common in forest nurseries in some parts of North America, particularly along the Pacific Northwest coast (Cooley and others 1985; Hansen and others 1980) and in nurseries in the Southeast (Bryan 1965; Hendrix and Campbell 1968). In general, species of *Phytophthora* are warm-weather fungi, adapted to moist conditions and usually cause problems in very wet or poorly drained portions of nurseries (Hendrix and Campbell 1968; Kenerley and others 1984). However, this requirement for warm, wet conditions can be tempered if enough inoculum and susceptible hosts are available (Banihashemi and Mitchell 1976). Douglas-fir may be susceptible to several species of *Phytophthora* along the Pacific coast (Hamm and Hansen 1987; Hansen and others 1980), and species of southern pine may be especially damaged by *P. cinnamomi* in the Southeast (Bryan 1965). Inoculation experiments with five species of *Phytophthora* (Campbell and Hamm 1989) indicated that pine, cedar, larch, and spruce species were generally tolerant to disease whereas some true fir and hemlock species were quite susceptible. Western larch apparently has not previously been reported as a natural host of *Phytophthora*.

Phytophthora cactorum has a very wide host range (Hardy and Sivasithamparam 1988; Keen and Yoshikawa 1983; Madden and others 1991; Pettitt and Pegg 1991). This species is especially well known causing basal cankers on several different types of fruit trees (Aldwinckle and others 1975; Bielenin and Jones 1988; Haygood and others 1986; Jeffers and others 1982; Wilcox and Ellis 1989). It has also been reported causing cankers on oaks (Mircetich and others 1977), Russian-olive (Carter 1953), and crown rot of Douglas-fir and true fir seedlings (Adams and Bielenin 1988). Pathology reported on some of these hosts is similar to that seen on the western larch at Coeur d'Alene, i.e., the fungus attacks and is restricted to the root crown and affected trees are essentially girdled by the fungus which progressively kills the cambium.

Once a tree is infected with *P. cactorum*, there is usually little that can be done to save it. The pathogen is quite aggressive and develops rapidly in a susceptible host. However, level of disease (overall number of diseased trees) within the tree improvement plantation was low. Isolated individual trees or small groups of trees were affected, but disease incidence did not significantly increase throughout the second or third growing seasons within the plantation. Limited disease increase and spread might have been due to the limited amount of soil-borne inoculum present (Mitchell 1978). High levels of *Phytophthora* were not detected in plantation soil and were probably limited by the tempering activity of *Trichoderma* and perhaps other antagonists in the soil. Susceptible hosts probably acted as baits for the fungus and because the overall soil was not very conducive to the pathogen or hosts were not extensively stressed (Hoitink and others 1986; MacDonald 1982), large-scale damage was not forthcoming.

Soil-borne pathogens including *Phytophthora* spp. may be greatly reduced by soil fumigation (James 1989; Munnecke and others 1974). The Coeur d'Alene Nusery commonly fumigates production areas with dazomet prior to sowing. This fumigant is generally effective in reducing populations of pathogens, at least in the upper portions of soil (James and others 1990). The tree improvement plantation site had been fumigated prior to planting of seedlings, but apparently not all pathogen inoculum had been eliminated or some had been reintroduced by the time disease became apparent. It is important that the organisms first reintroduced into fumigated soil not be pathogens; preferably they should be organisms competitive with or antagonistic toward pathogens (Munnecke 1984). Soil in the southwestern portion of the nursery where the plantation was established is relatively heavy (more silt and clay and less sand) compared with other parts of the nursery. Such a soil will have a greater opportunity to support populations of *Phytophthora* than more well-drained soils (Kenerley and others 1984). When water mold problems are suspected, spot treatments with fungicides such as metalaxyl, fosetyl-Al, and ethazole may help reduce problems (Benson 1984; Bielenin and Jones 1988; Cooley and others 1985; Rana and Gupta 1984). Such treatments have been especially effective in poorly drained portions of seedbeds where water tends to accumulate (Cooley and others 1985; Hamm and others 1984). However, broad-scale application of fungicides is discouraged because of environmental contamination problems, costs, and questionable efficacy (Cooley and others 1985).

Several successful attempts to control *P. cactorum* with non-chemical treatments have been reported. Biological control using the bacteria *Enterobacter aerogenes* and *Bacillus subtilis* (Gupta and Utkhede 1986; Utkhede 1987; Utkhede and Smith 1991) and hardwood bark soil amendments (Ellis and others 1986) have proven successful in fruit tree orchards.

It is unknown how *P. cactorum* was introduced into the Coeur d'Alene Nursery. It is possible the fungus has been present, at least at some level, for some time but has gone undetected because of its limited impact. It is also possible that the fungus was introduced into the nursery on infected plant material, although possible sources are unknown. Trees within the plantation were initially grown in greenhouses at the nursery. *Phytophthora* spp. have never been detected on container conifer seedling stock at this or other nurseries in the inland Northwest, although these pathogens have been previously reported in container nurseries (Hardy and Sivasithamparam 1988). Ornamental plants from residences adjacent to the affected plantation may have been an inoculum source as well, although no evidence of diseased materials on the edge of these residences bordering the nursery was found. Another possible inoculum source is irrigation water (Cooley and others 1985), although the nursery uses water from deep wells not likely contaminated with fungi such as *Phytophthora*. Therefore, sources of inoculum of this fungus cannot currently be determined.

Since this disease was initially diagnosed in 1989, the affected plantation fulfilled its usefulness as an early selection trial. Selected stock were used for vegetative propagation of progeny for further tests in the tree improvement program. All remaining trees were either removed or destroyed and the plantation site is currently in a fallow condition following incorporation of a corn cover crop in 1992. Recent soil assays indicated very high levels of *Fusarium* and *Pythium* as a result of incorporating large amounts of organic matter from the corn cover crop. Levels of *Pythium* were so great that the slower growing *Phytophthora* could not be detected on soil dilution plates. Therefore, it is unknown how extensive this pathogen currently occurs within the field. Growers plan to fumigate the area with dazomet prior to planting an additional seedling crop.

Phytophthora cactorum is apparently adapted to conditions at the Coeur d'Alene Nursery and is able to elicit disease in susceptible seedling hosts. Therefore, a new group of soil-borne pathogens previously not considered present at the nursery may cause future problems. Western larch is susceptible to *P. cactorum*, and Douglas-fir, which is grown extensively at the nursery, is also probably susceptible to this and other *Phytophthora* spp. based on reports from coastal nurseries (Campbell and Hamm 1989; Hamm and Hansen 1987; Hansen and others 1980). Therefore, future disease evaluations should consider fungi from this genus as possible pathogens.

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