Identity and Pathogenicity of Species of Phytophthora 
Causing Root Rot of Douglas-fir in the Pacific Northwest

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ABSTRACT


Three species of Phytophthora previously unreported from Douglas-fir were isolated along with P. cinnamomi from diseased trees from forest nurseries, forest outplanting sites, and seedling storage facilities in western Oregon and Washington. Two species were identified as P. cryptogaea and P. drechsleri on the basis of sporangial and colony morphology, temperature-growth relations, and by comparisons with isolates of known identity. A third species, designated Phytophthora sp. 1, did not correspond to any previously described species. Phytophthora cinnamomi and P. cryptogaea were highly virulent on dormant and growing Douglas-fir seedlings in greenhouse tests. Phytophthora drechsleri and Phytophthora sp. 1 appeared to be less virulent. Phytophthora cryptogaea, P. drechsleri and Phytophthora sp. 1 are similar to P. lateralis, a destructive pathogen of Port-Orford-cedar, in growth at low temperatures and should therefore be regarded as potentially dangerous forest pathogens in cool, moist sites.

Species of Phytophthora attack coniferous trees in nurseries, ornamental plantings, plantations, and forests throughout the world. Damping-off of young seedlings occurs in nurseries (8), and root rots develop in older plants in nurseries, outplantings, and natural forests. Phytophthora cinnamomi is the most widespread and destructive species on conifers (7, 8), but pathogenicity has also been proven for P. cactorum (5, 8), P. lateralis (21), P. boehmeriae (11), and several unidentified species (1, 19) isolated from conifers.

Phytophthora root rots of conifers in forests and field plantings are important in three regions. In the southeastern United States, the little leaf disease of shortleaf and loblolly pines (P. echinata and P. taeda), caused by P. cinnamomi (3, 24), retards growth and kills trees in forest sites with poor drainage and low fertility. Phytophthora cinnamomi also causes serious losses in plantation and shelterbelt plantings of P. radiata, Cupressus macrocarpa, and Chamaecyparis lawsoniana in New Zealand (9) and Australia (10). In southwestern Oregon, P. lateralis is causing an epidemic of root rot in native Port-Orford-cedar (C. lawsoniana) which threatens extinction of the tree as a commercial forest species (15, 18).

Phytophthora cinnamomi is the only species reported to cause root rot in Douglas-fir. It was first isolated from nursery seedlings and shown to be pathogenic in Portugal (4, 12). Crandall (4) found P. cinnamomi to be highly virulent on Douglas-fir and warned that it might cause serious damage to forests in the United States. Phytophthora cinnamomi was first discovered in the native Douglas-fir region of the Pacific Northwest in 1951 in ornamental plantings and nurseries (20). Roth and Kuhlman studied survival and infection under field conditions and concluded that P. cinnamomi was unlikely to constitute a threat to Douglas-fir forests because soil temperatures in winter and moisture levels in summer were too low for infection (16, 17).

In 1974 and 1975, Phytophthora root rots were observed in Douglas-fir seedlings and seed-orchard trees from five forest nurseries and from several regeneration sites planted with infested stock in Oregon and Washington. Phytophthora cinnamomi was isolated only from one orchard tree, while three other species previously unreported from Douglas-fir were isolated from diseased seedlings. This study was undertaken to determine the identity and to evaluate the pathogenicity of the three species of Phytophthora newly discovered on Douglas-fir.

MATERIALS AND METHODS

Phytophthora spp. were isolated by plating diseased root tissue of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] and by baiting with apple fruit (2). For plating, pieces of tissue up to 0.5 cm long or thick were excised from rotted roots and stems, surface-sterilized in 1% NaOCl for 1 minute, rinsed in sterile distilled water, briefly blotted dry on sterile filter paper, and plated on cornmeal agar (CMA) supplemented with 20 mg/liter pimaricin. After 1-5 days of incubation at 25 C, transfers were made from slow-growing (<= 1.5 cm of radial growth per day) colonies to CMA. In baiting attempts, portions of soil or roots (5 cm³) were packed into holes bored in fruits of apple (Malus sylvestris Mill. ‘Golden Delicious’). Holes were filled with water, surfaces were taped over, and apples were incubated at 25 C. After 5 days, apples were bisected and pieces of tissue from margins of areas with firm, dry rot were plated on CMA + pimaricin. Morphological and cultural characteristics of Phytophthora isolates were determined after growth on CMA and clarified V-8 juice agar (V-8A) at temperatures.
of 5-35°C for up to 30 days. Sporangia were obtained in colonies grown 4-5 days in pea broth (21) and then flooded with filtered soil-extract water for 24-96 hours.

To determine pathogenicity of Phytophthora isolates, both dormant and growing Douglas-fir seedlings 6-12 months old, with stems and roots 12-15 cm long, were grown in infested greenhouse soil in pots of 800 cm³ capacity. Inoculum consisted of isolates grown for 2 weeks on a mixture of cornmeal and sand (13) or of colonies (9 cm diameter, grown in V-8A) fragmented in water (10 colonies/300 cm³ water) for 60 seconds with a Sorvall Omnimixer. Cornmeal-sand inoculum was mixed with soil prior to transplanting of seedlings; inoculum of blended colonies was poured into wells in soil adjacent to roots after transplanting (approximately 45 cm³ inoculum per pot). Seedlings also were inoculated with 0.5 × 1.0-cm portions of infested agar from margins of colonies on V-8A taped onto lightly scraped areas of taproots. All seedlings were maintained in the greenhouse at 20-25°C under cool-white fluorescent lights with a 16-hour photoperiod for 3 months or until death of plants.

Severity of disease in a seedling was rated with a score of zero if 0-10% of the taproot was rotted, 1 if 10-40% was rotted, 2 if 40-70%, and 3 if 70-100%. If more than 75% of the circumference of a taproot was girdled by rot at any point, all of the distal root was considered to be rotted.

Phytophthora spp. were reisolated from plants with a disease score of 1 or more by surface sterilizing six pieces of tissue from taproots or large lateral roots and plating on CMA + pimaricin.

RESULTS

Symptomatology of root and stem rot.—Phytophthora root rots were observed in Douglas-fir seedlings up to 3 years old in nurseries and outplanting sites and in plants stored at low temperatures. Older stock was unavailable for examination. In nurseries, symptoms were most severe in trees growing in heavy soils, low areas of fields with poor drainage, and along drainage contours. Diseased stands were often sparse, with many seedlings dead and completely defoliated or with bronzed to light-brown needles. Crowns of plants at perimeters of disease pockets were stunted, chlorotic and partially defoliated. Infected dormant plants often did not break dormancy in the spring, or crowns became necrotic soon after swelling of buds or emergence and wilting of new shoots.

Roots of most dormant plants with severe top symptoms were completely rotted. Root rot also was severe in some dormant plants with only slight top symptoms. Root masses were reduced and feeder roots

Fig. 1. Root and crown symptoms of Phytophthora root rot in Douglas-fir seedlings following breaking of winter dormancy. Seedlings grown in soil infested with (left to right): Phytophthora cryptogea, P. drechsleri, P. cinnamomi, P. lateralis, and noninfested (control).
were often absent (Fig. 1). Outer cortical and periderm tissues were easily stripped from diseased feeder roots, and inner vascular tissues were discolored yellow to reddish-brown. Borders between rotted and healthy tissues, in cross-section, were either sharply delineated, or characterized by irregular, fine streaks of discoloration. Discoloration was usually present throughout roots less than 3 mm in diameter and restricted to cortex, periderm and phloem tissues in larger roots. However, in some seedlings, border zones of discoloration extended completely through taproots up to 1.5 - 2.0 cm thick. Large lateral and taproot were girdled when lesions developed in the vascular tissues at points of origin of decayed fine roots.

New roots which developed from infected plants breaking dormancy in the spring were often quickly rotted. In field plants, symptoms of chlorosis, necrosis and stunting in new shoots developed soon after most new roots were destroyed. In greenhouse-grown plants, similar symptoms often did not appear until both new and old roots were destroyed.

Root symptoms differed between nurseries. In some stands, feeder and small lateral roots of diseased seedlings were largely destroyed, but infection on taproots was restricted to small lesions and most plants remained alive. In other stands, many seedlings were killed by rot throughout the root systems.

When infected dormant seedlings were stored in bundles at low temperatures (2-6 C), Phytophthora storage roots developed in lower stems. Plants were girdled and killed by light-brown, water-soaked decay of cortical, phloem, and cambial tissues.

**Isolation of Phytophthora spp.**—**Phytophthora** spp. were isolated from diseased Douglas-fir seedlings from five forest nurseries in western Oregon and Washington, from one regeneration site, and from dormant seedlings in low-temperature storage. Isolates were obtained by the apple-baiting technique and by plating of diseased tissue. The apple technique was only successful in isolations from roots. When soil from a nursery field where trees were severely diseased was used in apple baits, only *Pythium* spp. were obtained among sixteen pythiaceous isolates from rotted apple tissue. When diseased roots of plants from the same field were used in apples, twelve *Phytophthora* and three *Pythium* isolates were obtained.

**Phytophthora** spp. were most easily isolated from diseased roots by tissue plating. *Phytophthora* colonies were often obtained from 100% of plated root pieces from greenhouse-infected plants, but never from more than 20% of root or stem pieces from field plants. Isolations from field plants were most successful in winter and early spring and seldom successful in summer.

Colonies of *Pythium* spp. usually developed within 24 hours on isolation plates, while hyphae of *Phytophthora* often did not appear in the agar until 24-48 hours. *Pythium* colonies frequently developed from fine roots of field plants but were seldom obtained from discolored vascular sections from which outer tissues had been removed.

**Identity of Phytophthora isolates.**—Four species of *Phytophthora* were identified among 37 isolates from plants from three nurseries. Identifications were made by the key of Waterhouse (22) and by reference to original (23) and secondary species descriptions. One isolate from an orchard tree was identified as *P. cinnamomi* Rand. on the basis of arbouscular fine hyphae and clusters of chlamydospores on CMA and V-8A, and by production of nonpapillate, proliferous sporangia on cultures in soil-extract water. Growth was slight at 10 C and did not occur at 5 C.

The remaining 36 isolates from seedlings were distributed among three species not previously reported from Douglas-fir. Seven isolates from one nursery were identified as *P. cryptogea* Peth. & Laf. All *P. cryptogea* isolates grew at temperatures of 3-30 C, and radial growth on V-8A was faster than for isolates of all other species at 30-50 C. Colonies on V-8A at 20-25 C were moderately to densely aerial and homogeneous or with faint ray or star patterns. No reproductive structures were produced on CMA or V-8A at any temperature. Numerous nonpapillate, proliferous sporangia developed on pea broth colonies after 18-24 hours in soil-extract water.

Fourteen isolates from two nurseries were identified as *P. drechsleri* Tucker. Growth occurred at 5-30 C; radial growth rates on CMA and V-8A were less than 30% of growth of *P. cinnamomi* and *P. cryptogea* at 20-25 C, and *P. drechsleri* isolates could be separated on that basis. On V-8A, isolates of *P. drechsleri* formed slight aerial mycelium and dense rosettes of sharp, compact "petals". This cultural pattern contrasted with the homogeneous colonies or weak patterns formed by *P. cinnamomi* and *P. cryptogea*. Some isolates of *P. drechsleri* produced hyphal swellings and vesicles similar to those described (6, 23).

Fifteen isolates, designated "Phytophthora sp. I", obtained from two nurseries in Oregon and Washington, could not be referred to previously described species (22, 23). Radial growth rates of isolates of *Phytophthora* sp. I were less than for *P. drechsleri* at all temperatures, and little growth occurred at 30 C. No colony patterns developed on agar media. Few to numerous oogonia with paragamous antheridia were produced on CMA and V-8A. Most oospores aborted. Sporangia were rare on agar media but frequent to numerous after 3-4 days of growth in soil-extract water. Most sporangia were subglobose to ovoid to ampulliform with thickened papilla, similar to sporangia of *P. bohmeriae* (6, 23), but intercalary and irregular sporangia were frequent. *Phytophthora* sp. I differed from *P. bohmeriae* in that antheridia were not amphigynous, and from *P. cactorum* in that sporangia were infrequent on agar, were not ellipsoidal to ovoid or deciduous, and were not borne in regular sori (22).

Oregon isolates of *P. cryptogea*, *P. drechsleri* and *P. cinnamomi* were compared with A1 and A2 isolates of *P. cryptogea* (P187 and P635), *P. drechsleri* (P208 and P209), and *P. cinnamomi* (Pe40 and Pe97), and an A1 isolate of *P. cambivora* (P601), received from D. C. Erwin, University of California, Riverside. Oregon and Riverside isolates of each species were similar in hyphal and colony morphology and temperature-growth relations. Shapes and sizes of sporangia of Oregon and Riverside isolates of each species were generally similar; however, differences in morphology of sporangia were not sufficient for use in separation of these species. *Phytophthora cryptogea* isolates from Oregon grew faster than Riverside isolates at 5-15 C on CMA but not on V-8A. The A2 isolate of *P. cryptogea* formed weak stellate patterns on V-8A identical to those formed by
several Oregon isolates, while colonies of the A1 isolate were homogeneous. Hyphal swellings similar to those figured by Waterhouse (23) were formed by Riverside isolates in soil-extract water, but not by Oregon isolates. *Phytophthora drechsleri* isolates from Oregon grew faster than Riverside isolates at 5-15 C, but slower at 20-30 C, on CMA. Hyphal swellings and vesicles were not observed in isolates of *P. drechsleri* from Riverside.

The A1 and A2 isolates of *P. cinnamomi*, *P. cryptogea* and *P. drechsleri*, and A1 isolate of *P. cambivora*, and one or more isolates of all species of Oregon and Washington were intercrossed on CMA and V-8A (+ and − 30 mg/liter cholesterol) at 25 C. Sexual structures formed only in crosses between Riverside isolates. Oogonia with or without oospores formed in intraspecific A1 × A2 crosses of *P. cinnamomi* and *P. drechsleri* and in several interspecific crosses of all species on V-8A with and without cholesterol. None of the Oregon and Washington isolates formed sexual structures in any intra- and interspecific crosses with themselves or with any Riverside isolate.

**Pathogenicity of Phytophthora spp. to Douglas-fir.**—Single isolates of each of the four species from Douglas-fir, and an isolate of *P. lateralis* Tucker and Milbrath from Port-Orford-cedar, were tested for pathogenicity on dormant and growing Douglas-fir seedlings. Dormant seedlings, transplanted into soil infested with cornmeal-sand inoculum at ratios of 1/8, 1/32, and 1/128 (v/v) or with slurries of blended cultures, were maintained in outdoor cold frames for 6 weeks (December 1974 to January 1975) and then transferred to the greenhouse. Growing seedlings, inoculated with blended cultures or agar blocks on taproots, were maintained continuously in the greenhouse.

Root and crown symptoms which appeared on seedlings 2-12 weeks after inoculation or transfer to the greenhouse were similar to those observed in field plants (Fig. 1). When taproots were more than 50% rotted, usually few or no lateral roots remained uninfeccc. Crown symptoms in plants inoculated in the greenhouse were less severe than in the field plants. *Phytophthora cinnamomi*, *P. cryptogea*, and *P. drechsleri* were reisolated from over 90% of diseased inoculated seedlings, whereas the slow-growing *Phytophthora* sp. 1 and *P. lateralis* were reisolated from less than 50% of diseased seedlings.

Significant differences (*P = 0.05*) in severity of taproot rot were observed between plants inoculated with different *Phytophthora* spp. (Table 1). *Phytophthora cinnamomi* and *P. cryptogea* caused more root rot than *P. drechsleri* at the two highest levels of cornmeal-sand inoculum and in dormant plants inoculated with blended cultures. *Phytophthora cinnamomi* also caused more disease than all other species at the lowest level of cornmeal-sand inoculum and in growing seedlings inoculated with blended cultures. The species of *Phytophthora* from Douglas-fir all caused more root rot than *P. lateralis* when seedlings were inoculated with infested agar blocks applied to injured taproots. Roots of two seedlings inoculated with *P. lateralis* by this method were rotted through at points of inoculation, but upward spread of infection was limited by formation of callus tissue. Restriction of root rot by callus formation was never observed in seedlings inoculated with *Phytophthora* isolates from Douglas-fir.

Ten growing seedlings were inoculated by incorporating into soil pieces of diseased roots (10 g/pot) from field plants from which both *P. cryptogea* and *P. drechsleri* had been isolated. Nine plants developed severe root rot within three months; *P. cryptogea* was isolated from eight plants and both species were isolated from one plant. When ten seedlings were inoculated with blended cultures of both *P. cryptogea* and *P. drechsleri*, only three developed root rot; *P. drechsleri* was isolated from two and *P. cryptogea* from one.

**DISCUSSION**

This study shows that three species of *Phytophthora* previously unreported on Douglas-fir cause root rots of seedlings in the Pacific Northwest. To date, disease has been observed only in seedlings in nurseries, storage facilities, and in the planting stock on a few regeneration sites. The extent to which any of the species newly discovered on Douglas-fir can survive, cause infection, and spread in forest sites is not known.

**TABLE 1. Severity of root rot caused by five species of Phytophthora in dormant and growing Douglas-fir seedlings with different inoculation methods and inoculum levels**

<table>
<thead>
<tr>
<th>Plant condition, inoculation method, inoculum level, and disease scoreb</th>
<th>Dormant plants</th>
<th>Growing plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cornmeal-sand ratio (v/v)</td>
<td></td>
</tr>
<tr>
<td>Speciesa</td>
<td>1:8</td>
<td>1:32</td>
</tr>
<tr>
<td>No inoculum</td>
<td>0.0 A</td>
<td>0.0 A</td>
</tr>
<tr>
<td><em>P. lateralis</em></td>
<td>0.0 A</td>
<td>1.3 B</td>
</tr>
<tr>
<td><em>P. cinnamomi</em></td>
<td>2.8 C</td>
<td>2.6 C</td>
</tr>
<tr>
<td><em>P. cryptogea</em></td>
<td>2.8 C</td>
<td>2.6 C</td>
</tr>
<tr>
<td><em>P. drechsleri</em></td>
<td>1.9 B</td>
<td>1.7 B</td>
</tr>
<tr>
<td><em>Phytophthora</em> sp. 1</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

aIsolates of all species obtained from Douglas-fir except *P. lateralis* from Port-Orford-cedar. One isolate of each species was used in all inoculations.

bPlants dormant or growing at time of inoculation. Infested cornmeal-sand mixture and blended colonies were used to infest soil. Infested agar blocks were applied directly to wounded taproots. Disease score: 0 = little or no taproot rot, 3 = severe taproot rot. Plants were scored after 3 months in a greenhouse at 20-25 C. Values for agar-block inoculations = means of disease scores of five seedlings; all other values = means of scores of ten seedlings. Values not followed by the same letter within a column are significantly different (*P = 0.05*) as determined by Duncan's multiple range test.
Single isolates of the three species and of *P. cinnamomi* appeared to differ in virulence to Douglas-fir. In inoculated plants, *P. cinnamomi* and *P. cryptogea* were most virulent while *P. drechsleri* and *Phytophthora* sp. 1 appeared to be intermediate in virulence. *Phytophthora lateralis*, a species not known to attack Douglas-fir under natural conditions, was weakly virulent or avirulent. However, apparent differences in virulence may have been partly due to use of inoculation methods which favored one or more species. *Phytophthora cryptogea* and *P. drechsleri* caused severe disease when inoculum of infected root pieces was incorporated into soil, but little disease when inoculum consisted of blended colonies. Similarly, *P. cinnamomi* caused more disease than *P. cryptogea* in two trials with inoculum of cornmeal-sand and blended cultures, but this species formed numerous chlamydospores in both inocula, and these could have survived longer and caused more infection in soil than the hyphal fragments in inoculum of *P. cryptogea*. When infected agar blocks were applied to taproots, significant differences in virulence of species from Douglas-fir were not observed.

The potential of *Phytophthora* spp. to cause disease in forest sites is related to their pathogenicity and to their capacity to survive and cause infection under field conditions. *Phytophthora lateralis* is highly virulent on Port-Orford-cedar and also grows and infects at low temperatures (less than 5 C) (21). This species spread to the native cedar forests of the Coastal Range and initiated an epidemic within 15 years after it first appeared in nurseries and ornamental plantings in the Willamette Valley in Oregon (18). *P. cinnamomi*, in contrast, has been known in Oregon nurseries for over 25 years, but has never been found causing disease in Douglas-fir or other susceptible hosts in forest sites. Roth and Kuhlman showed that although *P. cinnamomi* could survive in forest sites, infection did not occur below 15 C, and forest soil temperatures remained well below that level during the 7- to 8-month rainy season when soil moisture would have been sufficient for infection to occur (14, 16, 17).

*Phytophthora cryptogea*, *P. drechsleri*, and *Phytophthora* sp. 1 are similar to *P. lateralis* in growth at low temperatures and are also moderately to highly virulent on Douglas-fir. These species, unlike *P. cinnamomi*, should therefore be regarded as potentially dangerous forest pathogens in cool, moist sites in the Pacific Northwest. Further studies are needed to determine whether survival and infection occur at low temperatures, to determine host ranges, and to further assess pathogenicity of these species to Douglas-fir.

**LITERATURE CITED**


