Survival, Spread, and Pathogenicity of *Phytophthora* spp. on Douglas-fir Seedlings Planted on Forest Sites

E. M. Hansen, L. F. Roth, P. B. Hamm, and A. J. Julis

Assistant professor, professor, graduate research assistant, and research associate, respectively, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

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ABSTRACT

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Douglas-fir seedling stock infected in the nursery with *Phytophthora cryptogea, P. drechsleri, P. megasperma, P. cactorum,* and an unidentified *Phytophthora* sp. were outplanted on commercial forest sites to test survival of the diseased trees and of the pathogens. Mortality of trees initially classified in severe, moderate, and inconspicuous symptom classes at outplanting reached 61, 26, and 11%, respectively, after 18 mo. *Phytophthora* was recovered about equally from roots of trees in each symptom class (15, 13, and 12%). Surviving trees regenerated healthy roots above old lesions even though *Phytophthora* persisted. Disease spread was limited. None of 360 healthy trees planted 0.6 m downslope from diseased trees became infected, and only 2 of 720 healthy trees became infected after each was paired with a diseased tree in the same planting hole.

Species of *Phytophthora* damage forest trees in several areas of the world and may induce greater damage as human activities disperse the fungi to new areas. Although agricultural commerce and cultural practices have led to widespread infestation of cultivated land by *Phytophthora* spp., most forested areas of the Pacific Northwest are not yet contaminated. The susceptibility of most native forest vegetation to various species is unknown, however, and circumstances affecting the spread of *Phytophthora* onto forest lands must be viewed with concern.

Recognition of *Phytophthora* as causal agent of root rot on seedlings of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) in forest tree nurseries in the Pacific Northwest (3) prompted this study of the persistence and spread of *Phytophthora* spp. on forest sites. *Phytophthora drechsleri* Tucker, *P. cryptogea* Peth. & Laf., *P. megasperma* Drechsler, *P. cactorum* (Leb. & Cohn) Schroet., and an unidentified *Phytophthora* spp. 1 were isolated from dead and dying bare-rooted stock in nurseries (2,3). About 150 million seedlings are transplanted annually to forest sites in Oregon and

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Washington, providing ample opportunity for the introduction of the pathogen from infested nurseries to commercial forest land. The current work was spurred in 1975 by reports of extensive mortality among seedlings transplanted from infested nurseries to forest sites.

MATERIALS AND METHODS

Infected Douglas-fir seedlings from three nurseries were transplanted to different forest sites 1–2 yr after clearcutting. Twelve planting sites were located in three areas of western Oregon: the western Cascade Range, the central Coast Ranges, and southwest Oregon. To reduce the possibility of introduction of pathogens into uncontaminated forest land, infected trees were planted only on sites previously planted to trees from the same nursery. Infected seedlings were obtained from nurseries managed by the Oregon Department of Forestry (State), the USDA Forest Service (F.S.) and a private corporation (private). One experimental site was established in each of the three geographic areas with trees from the State and F. S. nurseries; two sites in each area were planted from the private nursery (Table 1).

Infected seedlings (2 yr seedbed, 1 yr transplant) with crown symptoms were dug from the nurseries and graded into severe, moderate, or inconspicuous root symptom classes. Control seedlings were either bare-root (2 yr in seedbed) stock from a disease-free nursery or healthy container-grown seedlings.

Three adjacent plots (designated A, B, and C) were planted on each of the 12 experimental sites. Plot A was designed to assess mortality of infected stock and to monitor fungus spread. Planting spots for 60 nursery-infected trees were 0.5 m apart in rows 1 m apart, oriented along the contour. Twenty trees each of the three symptom classes were randomly assigned to the planting spots and a healthy container-grown seedling was planted in the same hole as each infected tree; a second healthy tree was planted 0.6 m downslope from alternate infected trees.

Plot B was planted with 40 healthy bare-root trees and 10 healthy container-grown trees to check seedling mortality induced by agents other than *Phytophthora* and to detect preexisting *Phytophthora* infestation. It was located to avoid possible spread from infected trees in Plot A.

Plot C was used to determine the time of fungus survival. It contained from 15 to 40 naturally infected trees, the number in each plot depending on availability of plants with symptoms.

Mortality was recorded for Plots A and B at each site after 3, 6, 12, 18, and 24 mo. Dead trees on Plot B and one-fourth of the trees on Plot C were dug for laboratory isolation of *Phytophthora* spp. on each of the first four sampling dates. After 24 mo, the remaining trees on Plots A and B were dug for isolation. Soil temperature and water content at 10–15 cm were determined at each sampling date (Table 1). Root wet weights were recorded and isolations from all trees were attempted after 24 mo. Prior to multivariate analysis of variance, data were standardized to zero mean and unit variance.

Phytophthora spp. were isolated from roots by direct plating onto selective corn meal agar, CMA-PVP (20 mg/L pimaricin, 200 mg/L vancomycin and penicillin G), and by baiting with apples (2). Only direct plating was used for isolation from samples collected after 0 and 3 mo. For later samples, a portion of roots from naturally infected trees were plated directly on CMA-PVP and the remaining roots were plunged into whole apples. Control trees (which were healthy when transplanted) that had root lesions were treated similarly, but when no lesions were present on these trees, only apple baiting was used. When plating on CMA-PVP, five to 25 root pieces 2–5 mm long were cut from lesions margins of each seedling, surface sterilized in 1% sodium hypochlorite, rinsed in distilled H_2O, blotted dry, and placed on CMA-PVP. For apple baiting, root samples from each seedling were inserted into three apples per seedling and incubated 1–2 wk at 20–22 C. Incubated apples were cut open, and isolations were made by transferring small bits of apple tissue from the margins of rotted areas to CMA-PVP. All plates were incubated at room temperature and examined daily. Suspected *Phytophthora* colonies were transferred to either CMA or CMA-PVP for confirmation.

TABLE 1. Physical characteristics of Douglas-fir experimental sites 3, 6, 12, and 18 mo after transplanting

Site identification[a]	Elevation (m)	Slope aspect	Soil pH	Soil temperature (C) 3 mo	6 mo	12 mo	18 mo[b]
Southwestern Oregon plots							
Private 1	200	90% S	4.8	19	16	10	13
Private 2	200	5% W	5.0	20	18	11	14
State 1	80	60% N	4.6	19	16	13	11
F.S. 1	1,300	60% NW	4.9	18	17	7	5
Coast Range plots							
Private 3	100	5% NW	4.7	17	15	9	12
Private 4	100	5% S	4.5	16	16	9	12
State 2	300	5% W	4.9	16	16	9	12
F.S. 2	300	5% NE	4.6	17	14	8	12
Cascade Mountains plots							
Private 5	300	5% N	5.4	19	19	11	6
Private 6	300	5% E	5.5	19	18	11	6
State 3	300	30% N	5.6	19	17	10	5
F.S. 3	300	5% W	5.2	18	12	9	9

[a] Origin of stock and site number. Private nursery, State Forestry nursery, Forest Service (F.S.) nursery.
[b] 3, 6, 12, and 18 mo after outplanting, March 1976.

RESULTS

Tree mortality. Mortality of naturally-infected stock from all nurseries was directly related to the root rot rating at the time of transplanting (Table 2). After 18 mo, combined mortality for all sites was significantly different ($P = 0.05$) for trees with severe symptoms (61%), moderate symptoms (26%), and inconspicuous symptoms (11%). Control trees (Plot B) averaged 15% mortality. Many dead trees, originally infected in the nursery, had *Phytophthora* lesions girdling the main roots. Most mortality (71% of final mortality) occurred within 3 mo of outplanting. Total mortality of nursery infected trees (Plot A) and control trees (Plot B) on southwestern Oregon sites (41 and 26%), exceeded losses on sites in the Coast Ranges (28 and 11%) and in the Cascades (29 and 7%).

Root weight (after 24 mo) of surviving nursery-infected trees was related to initial disease rating and nursery of origin, but the differences were not significant ($P = 0.05$, Table 3). Height growth in 1977 also increased with disease rating but was variable due to

TABLE 2. Mortality after 18 mo of nursery infected Douglas-fir trees and of initially healthy trees planted together with and 0.6 m downslope from infected trees

Nursery	Symptom rating[a]	Mortality (%) Infected trees	Initially healthy trees: Same hole	Initially healthy trees: 0.6 m Downslope	Control Trees (Plot B)
Private	1	63	8	8	
	2	25	7	8	
	3	11	7	15	14
State	1	83	10	13	
	2	33	10	10	
	3	13	12	10	21
Forest Service	1	35	8	7	
	2	20	7	7	
	3	10	3	3	11

[a] Symptoms at time of transplanting: 1 = severe root rot symptoms, 2 = moderate symptoms, and 3 = inconspicuous symptoms.

animal browsing.

Fungus spread. *Phytophthora* did not spread appreciably in the field. After 24 mo only 2 of 720 initially healthy container-grown trees planted in the same hole with nursery infected trees, and none of the initially healthy trees planted downslope from infected trees became infected. Mortality of healthy trees planted along with infected trees was no greater than was the mortality of trees planted 0.6 m downslope or of control trees in Plot B (Table 2).

***Phytophthora* spp. survival in roots.** Apple baiting and direct plating were equally efficient for recovering all *Phytophthora* spp. except *Phytophthora* sp. 1, (83% of isolates by direct plating), but using both techniques together nearly doubled the total number of recoveries. *Phytophthora* spp. were recovered from 134 naturally infected trees subjected to both isolation methods. Fifty trees yielded *Phytophthora* isolates by apple baiting only and 64 by direct plating only. *Phytophthora* was recovered from only 20 trees by both techniques.

Recovery of *Phytophthora* spp. from nursery-infected trees was greatest (38% of trees sampled) after 12 mo, and declined to 10% after 24 mo (Table 4). Isolation success did not vary with season of the year, and was similar for living and dead trees after 12 and 18 mo. Some of the trees sampled had been dead for up to 6 mo. The 24-mo sample was collected from Plot A. Many of these had died shortly after outplanting and isolation success from dead trees was much lower at this time. Living trees from which *Phytophthora* was recovered after 24 mo lacked conspicuous foliar symptoms, and root weight and height growth were not significantly different from trees not yielding *Phytophthora*. Root symptoms were present on all trees from which *Phytophthora* spp. were isolated.

At the time of planting isolation success from trees with severe, moderate, and inconspicuous symptoms was similar (16, 15, and 14%) to isolation from trees living after 24 mo (15, 13, 12%).

All *Phytophthora* spp. previously found in the nurseries (2) were recovered from nursery-infected trees after 24 mo on the forest sites. *P. drechsheri* was isolated more frequently than other species from trees with severe or moderate symptoms (50% of isolates) but *Phytophthora* sp. 1 predominated on trees with inconspicuous symptoms (75% of isolates).

Phytophthora was isolated from 1 of 452 Plot B control trees alive after 24 mo. The isolate was distinct from any previously recovered from Douglas-fir. It was homothallic, with amphigynous antheridia and sympodially branched, nonpapillate sporangia. Control trees which died earlier did not yield *Phytophthora*.

TABLE 3. Root fresh weight and 1977 height growth of nursery infected Douglas-fir trees surviving after 24 mo in the field

Nursery	Disease rating[a]	Root weight (g)	Height growth (cm)	Trees (no.)
Private	1	23.9	13.2	27
	2	36.1	17.4	46
	3	51.2	20.8	54
State	1	23.3	12.1	10
	2	44.6	21.2	35
	3	72.3	22.4	51
Forest Service	1	7.1	6.7	24
	2	9.0	7.8	30
	3	14.8	11.3	39

[a]Symptoms at time of transplanting: 1 = severe root rot, 2 = moderate root rot, and 3 = inconspicuous root rot.

DISCUSSION

Certain *Phytophthora* spp. often are difficult to isolate. Recovery was exceptionally high in this study considering the small size of root systems available for sampling. Isolation by apple baiting was appreciably more successful than by direct plating because the entire root system was sampled. *Phytophthora* sp. 1 could not be recovered consistently from apples, however, presumably because of its slow growth (3).

The *Phytophthora* spp. transplanted from tree nurseries to forest sites in Oregon did not spread from originally infected trees under conditions prevailing in 1976 and 1977. The fungi survived on initially infected trees, but could be recovered from only 2 of 720 initially healthy trees planted with roots intermingling with those of infected trees.

The potential of *Phytophthora* spp. for damage in the forest appears to rest heavily on how well they are adapted to the forest environment. *P. cinnamomi,* although highly pathogenic to Douglas-fir, is poorly adapted to forest sites. Roth and Kuhlman (4) showed that forest soils were too cold in the winter wet season and too dry in the summer for *P. cinnamomi* to spread. *P. lateralis,* however, grows well at cooler soil temperatures (5) and now threatens the economic survival of Port Orford-cedar following its introduction to SW Oregon forests from ornamental nurseries. Like *P. lateralis,* the *Phytophthora* isolates obtained in the current study grew, sporulated, and infected Douglas-fir seedlings at temperatures well within the range encountered in field soils (A. J. Julis, *unpublished,* and Table 1).

Soil moisture was not adequately monitored in this study, but was certainly limiting to zoospore production much of the time. The winter of 1976–1977 was the driest on record at most sites. From September through December, normal rainfall at the Coast Range sites is 99 cm; only 23 cm fell during this period in 1976, and the drought continued into the spring. *Phytophthora* was recovered from 35% of the trees in October 1977, however, and the next year had normal rainfall, still with little or no fungus spread. Lack of spread to trees 0.6 m away may have resulted from limited zoospore movement (1) in well-drained forest soils, but trees with roots intertwined with diseased roots were seldom infected either.

Although *Phytophthora* spp. survived on outplanted trees, they were unable to colonize new roots. Most trees still infected with *Phytophthora* after 24 mo had produced new roots above old tap root lesions. Mortality of infected trees soon after transplanting was high, but trees that survived the initial transplant shock despite partially disfunctional roots effectively tolerated the fungus in most cases.

Mortality of outplanted trees was caused by many factors in addition to *Phytophthora,* including drought, animal damage, and

TABLE 4. Isolation of *Phytophthora* spp. from infected seedlings from three nurseries at transplanting and after 3, 6, 12, 18, and 24 mo on forest sites in western Oregon

	Isolation success (%)								
	0 mo	3 mo	6 mo	12 mo		18 mo		24 mo	
Nursery				All[a]	Dead[a]	All	Dead	All	Dead
Private	14(58)[b]	46(46)	32(44)	53(45)	50(24)	50(46)	54(13)	20(167)	11(38)
State	8(48)	20(15)	17(18)	29(17)	50(6)	25(12)	50(4)	7(125)	3(77)
Forest Service	20(60)	0(27)	27(30)	21(29)	40(5)	0(17)	0(6)	5(116)	4(23)
Total	14(166)	27(88)	27(92)	38(91)	49(35)	35(75)	39(23)	10(458)	5(138)

[a] Isolation success from both living and dead trees (all) and dead trees only (dead).
[b] Values in parenthesis are number of trees from which isolations were attempted.

poor planting. Nevertheless, the strong correlation between mortality and disease rating on all sites clearly demonstrates the hazard of outplanting diseased stock. Recovery of *Phytophthora* from only one Plot B control tree suggests the virtual absence of *Phytophthora* from forest soils, an observation supported by extensive soil sampling in western Oregon (unpublished data). The single isolate obtained in this study may have originated from a nursery infection, although the source nursery is not known to have *Phytophthora,* or it may have been introduced during logging or regeneration activities. *Phythium* spp., extremely abundant on seedlings before outplanting, were isolated infrequently at 3 mo and later sample times, and then only when clods of nursery soil remained attached to tree roots.

The *Phytophthora* isolates examined in this study, like *P. cinnamomi* previously (4), did not establish themselves in forest environments. Unfortunately, forest tree nurseries are increasingly located in agricultural areas and seedlings will continue to be exposed to pathogenic fungi. Rigid insistence on healthy stock and continuing surveillance will be necessary to protect the forest resource.

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