Testing Port-Orford-Cedar for Resistance to *Phytophthora*

E. M. HANSEN, P. B. HAMM, and L. F. ROTH, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331

ABSTRACT

Hansen, E. M., Hamm, P. B. and Roth, L. F. 1989. Testing Port-Orford-cedar for resistance to *Phytophthora*. Plant Disease 73:791-794.

Root rot mortality of Port-Orford-cedar (*Chamaecyparis lawsoniana*) in western Oregon and Washington and northwestern California is caused almost exclusively by *Phytophthora lateralis*. Port-Orford-cedar is also susceptible to *P. cinnamomi* in artificial inoculation but not to other *Phytophthora* species pathogenic on conifers in the Northwest. Progression of *P. lateralis* lesions was slower on four cedar trees selected for phenotypic resistance than on randomly selected trees. Inoculations of seedlings and rooted cuttings with zoospores or infested soil and of excised branches with inserted mycelium gave similar results on the selected trees. Branch testing of mature trees and zoospore spray inoculation of seedlings have advantages over other techniques.

Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) is a native conifer in the forests of southwest Oregon and northwest California and a valuable timber species. It is also widely planted as an ornamental in the Pacific Northwest and in other cool, moist parts of the world. In the 1950s, production of Port-Orford-cedar in nurseries growing ornamentals was curtailed, then nearly eliminated, by two serious root disease fungi, *Phytophthora lateralis* Tucker & Milbrath and *P. cinnamomi* Rands (1,15,17). Since that time, *P. lateralis* has spread through much of the native range of Port-Orford-cedar (7,12). Current spread in the forest is primarily associated with road construction and maintenance, vehicle traffic, timber harvest, and water movement downslope from disturbed areas (17). The consequence is ecological disruption in sensitive habitats, a multimillion dollar annual loss in timber revenue, and a serious threat to the commercial future of the species.

Since introduction of *P. lateralis* into the native cedar habitat, five additional species of *Phytophthora* have been found to attack conifers in the Northwest (5). Their pathogenicity to Port-Orford-cedar is largely unknown. Moreover, there is no proven resistance to *P. lateralis* within Port-Orford-cedar, although occasional trees remain alive after surrounding cedars have been

killed. L. F. Roth (*unpublished*) rooted cuttings from hundreds of such survivors and transplanted them to soil artificially infested with *P. lateralis*. Mortality was invariably high in these tests, suggesting that the survivors had somehow escaped disease, or that resistance of the test trees was overcome by artificially high inoculum loads, or that resistance was not well expressed by rooted cuttings.

The goal of the present work was to further the search for resistance in Port-Orford-cedar by: 1) determining which *Phytophthora* species are potentially involved by isolating the fungus from dead and dying Port-Orford-cedar trees, 2) developing inoculation systems for seedlings and mature trees that allow exposure to measured inoculum levels and assessment of rate of disease increase, and 3) evaluating additional phenotypically resistant trees.

MATERIALS AND METHODS

Isolation of *Phytophthora*. Recently killed and dying Port-Orford-cedar trees (103 in all) were located widely in the species' native range and in areas of Oregon where it has been extensively planted as an ornamental. Two methods—direct isolation and baiting—were used to recover *Phytophthora*.

In the first method, the fungus was directly isolated from the advancing margin of discolored phloem, usually located on the stem within 0.5 m of the ground. A 25-cm portion of tissue containing the margin was removed from each tree and kept cool until laboratory isolation was possible (<48 hr). The sample was split tangentially, exposing the margin. Pieces 2-3 mm square were cut from this region and transferred to cornmeal agar containing 20 μg/ml of pimaricin (CMP). *Phytophthora* colonies were evident after 2-4 days of incubation at room temperature in the dark.

In the second method, *Phytophthora* was baited from soil collected and composited from three locations around the base of each dying tree. Organic matter was separated from the samples by flotation (9), and 50-g (wet weight) subsamples were distributed among 10 cups in the double-cup baiting method (4,8). Five 2.5-cm lengths of Port-Orford-cedar foliage were floated as baits in each cup and after 6 days were transferred to CMP with only 10 g/L of agar and containing 200 μg/ml of vancomycin. Plates were observed microscopically after 5-6 days and *Phytophthora* colonies were identified (5). *Phytophthora* isolates were subcultured to assure purity and stored in liquid nitrogen.

Susceptibility of 6-mo-old Port-Orford-cedar seedlings to single isolates of eight *Phytophthora* species found on conifers in Oregon (5) was tested by transplanting to soil infested with CMS inoculum. The following isolates withdrawn from nitrogen storage were used: *P. lateralis* and *P. cinnamomi* recovered from dying Port-Orford-cedar trees in western Oregon, *P. pseudotsugae* Hamm & Hansen, *P. cactorum* (Leb. & Cohn) Schr., *P. drechsleri* Tucker, *P. cryptogea* Peth. & Laf., and *P. megasperma* Drechs. (BHR and DF types [6]). Twenty seedlings were inoculated with each fungus. Dead and declining seedlings were harvested at 2-wk intervals, and all remaining trees were harvested at 12 wk.

Stock for resistance testing. Ten mature Port-Orford-cedar trees were used as parent trees for most of the resistance testing. Trees 1-4, ranging in age from 10 to 20 yr, were growing close together in a *Phytophthora* experimental area on the Oregon State University (OSU) campus. Tree 1 was transplanted as a seedling from the Coos County (Oregon) Forest, and trees 2-4 originated from cuttings of trees in Coos County. Because all four parent trees had survived from age 2 yr in infested soil under conditions that killed hundreds of their contemporaries, they were considered phenotypically resistant to *Phytophthora* and are hereafter referred to as the "selected" trees. The remaining six trees—9, 10, CB11, CB12, CB13, and CB14—are termed the "unselected" trees.

Trees 9 and 10 were 20-yr-old ornamentals on the OSU campus, apparently unexposed to *P. lateralis*; trees CB11 and CB12 originated 10 yr ago as cuttings from unselected parents of unknown origin; and trees CB13 and CB14 were wild trees about 15 yr old and growing in Coos County.

Resistance to *Phytophthora* was tested via inoculation on terminal portions (about 1 m long) of branches detached from each of the 10 mature Port-Orford-cedar trees, on cuttings rooted from each mature tree and grown for 6–12 mo, and on seedlings grown from open-pollinated seed of trees 1, 2, and 9 in 22-ml tubes (18 × 110 mm) in the greenhouse for 6–18 mo. Two isolates of *P. lateralis* (366 and 632) originating from widely separated trees were used in most tests.

Stem inoculations (wounding). A longitudinal slit, about 1 cm long, was made in the stem of detached branches of mature Port-Orford-cedar trees (10 branches per parent), rooted cuttings (four to 20 per parent), and seedlings (20 per parent), and a 2-mm portion of mycelium from the margin of a colony in pea broth was inserted laterally between the phloem and the xylem of each stem. The wound was covered with petroleum jelly. Branches were 5 mm in diameter and cuttings and seedlings, about 2 mm, at the point of inoculation. The cut ends of branches were kept immersed in water for the duration of the experiment.

Successful inoculations resulted in an area of discolored, shrunken bark around the wound, which gradually expanded up and down the length of the stem. Uninoculated check wounds healed without discoloration. Disease was assessed after 2 wk for seedlings and cuttings and 8 wk for branches by measuring the length to which the lesion had extended from the limits of the initial wound.

Soil inoculations. Soil inoculum was prepared by growing the fungus in cornmeal-sand (CMS) for 4 wk (3,10) and then mixing one part of the infested CMS with 16 parts steam-pasteurized soil or with vermiculite. Rooted cuttings and seedlings were transplanted into infested soil or into greenhouse soil mix layered over CMS inoculum and randomized in the greenhouse. In the latter case, "book pots" (Spencer-Lemaire Industries, Edmonton, Alberta, Canada), hinged at the bottom, facilitated placement of roots above the CMS inoculum layer.

After incubation in the greenhouse for 4 wk (seedlings) or 8 wk (cuttings), stock was removed from the pots and carefully washed. Roots were examined for lesions, and the distance to the advancing lesion relative to the soil line was recorded (− if fungus had not yet reached the soil line, + if it had advanced beyond). Overall root disease was also

rated on a 0–5 scale, where 0 = 0–10% dead roots, 1 = 11–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%, and 5 = seedling dead. Infection was confirmed by direct isolation from surface-sterilized symptomatic roots to CMP medium or by baiting from the entire root system with cedar foliage baits.

Zoospore inoculation. Zoospore inoculum of *P. lateralis* was prepared by transferring five 3-mm disks from the margins of actively growing colonies on cornmeal agar to pea broth in standard petri plates. After 5 days' incubation, colonies were thoroughly rinsed in distilled water and flooded with soil extract water. Maximum numbers of zoospores were released after 16 hr of incubation in the dark at 15 C. The terminal 3 cm of the roots of cuttings and seedlings was immersed in a water suspension of zoospores ($2.7 \times 10^5/$ml concentration) for 24 hr. For cuttings, 10 from each of eight parent Port-Orford-cedar trees (selected trees 1–4 and unselected trees CB11–CB14) were inoculated; two cuttings per parent were uninoculated checks. For seedlings, 10 from each of three parents (selected trees 1 and 2 and unselected tree 9) were inoculated. After inoculation, cuttings and seedlings were transplanted to vermiculite in growth tubes, then kept in a greenhouse until lesions were visible at the soil line.

Alternatively, root tips of seedlings from parent trees 1 and 9 were sprayed with an atomized suspension of $5.6 \times 10^4/$ml or $1.5 \times 10^4/$ml of zoospores from isolates 366 and 632, respectively, of *P. lateralis*. Twenty seedlings per parent per isolate were tested. Seedlings were then incubated in a moist chamber for 4 days before being transplanted to vermiculite in growth tubes. Disease assessment and confirmation for both zoospore inoculations were as previously described.

Analysis. Where stock from two parent trees were compared, means were evaluated with Student's *t* test ($P = 0.05$). Where stock from three or more parent trees were compared, analysis of variance was used to identify tests with a significant *F* value, then Duncan's multiple range test ($P = 0.05$) was used to test individual means.

RESULTS

Pathogenicity of *Phytophthora* species to Port-Orford-cedar. *Phytophthora* was isolated in association with 72 of 103 (70%) dying Port-Orford-cedar trees in western Oregon. *P. lateralis* was recovered from 70 trees and *P. cinnamomi*, from two. In 48 trees, *Phytophthora* was recovered both by baiting and by direct isolation. Nine additional trees were positive only by baiting and 13, only by direct isolation. Both isolates of *P. cinnamomi* were from soil baiting.

P. lateralis quickly killed all 20

inoculated Port-Orford-cedar seedlings and was reisolated from each. *P. cinnamomi* killed six trees and was reisolated only from those trees. Single seedlings were killed by *P. megasperma* DF and *P. cryptogea*. Similar results were obtained when the test was repeated, except that *P. cryptogea* did not kill any seedlings.

Stem wounding. There were no consistent differences between the two *P. lateralis* isolates in reactions of seedlings, cuttings, or branches, so results were combined.

Symptoms developed most rapidly in seedlings where stems were girdled and starting to dry after 2 wk. In all three types of stock, lesion development was less on material from selected than on that from unselected trees (Table 1). Lesions on seedlings from selected tree 1 averaged 5.8 cm, compared with 8.7 cm on seedlings from unselected tree 9. Lesions on rooted cuttings from selected trees 1, 2, and 4 averaged 3.4 cm, compared with 7.8 cm on cuttings from unselected trees 10, CB11, CB12, and CB14 after 3 wk. Differences were even more pronounced on detached branches; lesions on branches from selected trees averaged 0.6 cm, compared with 6.3 cm on branches from unselected trees after 4 wk. No lesions developed on any wounded but uninoculated check trees. Branch inoculations were repeated three times with different combinations of selected and unselected trees. Results were similar, except during a summer test when high temperatures apparently inactivated the fungus.

Soil inoculations. Rooted cuttings planted above a layer of CMS inoculum showed patterns of disease development similar to those observed with the other inoculation methods. After 4 wk, 19% of the 48 cuttings from unselected trees 10 and CB11–CB14 were dead, compared with 6% of the 32 cuttings from selected trees 1 and 4. However, 15% of the 20 uninoculated check cuttings were dead as well; many of these dead trees had been severely root-pruned.

Lesions on cuttings from unselected trees extended further (to within 2.2 cm of the soil line) than lesions on cuttings of selected trees (6.5 cm below the soil line). Root rot ratings for check and selected trees were similar (Table 1). *P. lateralis* was isolated from one contaminated check cutting at the end of the study, from 47% of the cuttings from inoculated selected trees, and from 77% of the cuttings from inoculated unselected trees.

Zoospore inoculation. *Immersion.* Seedlings from selected trees 1 and 2 showed fewer symptoms of root rot than seedlings from unselected tree 9 (Table 1). Two seedlings from each selected tree showed no root lesions at all, and the average extent of the highest lesion on the remainder was 4.4 cm below the soil

line. Average root rot rating was 1.2. *P. lateralis* was recovered from 45% of seedlings from selected trees. Nine of 10 seedlings from unselected tree 9 were girdled; lesion extent averaged 1.7 cm above the soil line and root rot rating averaged 4.5. *P. lateralis* was recovered from all 10 seedlings from the unselected tree.

Results with rooted cuttings were confounded by the early death of 20 of the 96 cuttings, apparently the consequence of heavy root pruning necessary to fit the cuttings into the inoculation containers. *P. lateralis* was recovered from only two of the 20 cuttings, although 16 of the 20 were inoculated and from unselected trees. Results were computed for the cuttings that survived to the end of the study (5.5 wk). As a group, cuttings from the selected trees (1–4) performed better than those from unselected trees CB11–CB14, although the worst of the former (tree 2) was comparable in this test to the best of the latter (CB11). Three of 38 cuttings from selected parents were girdled; lesion extent averaged 5.4 cm below the soil line and root rot rating averaged 1.6 (Table 1). Fifteen of 24 cuttings from unselected trees were girdled; lesion extent averaged 0.6 cm above the soil line and root rot rating averaged 4.1. There was no evidence of infection on uninoculated check trees, and root rot rating averaged 0.2. *P. lateralis* was recovered from 58 of 62 inoculated cuttings from both sources. The test was repeated using a different combination of selected and unselected trees, and results were similar.

Spray. Reactions to the two isolates of *P. lateralis* were similar, so results were combined. Two of 40 seedlings from selected tree 1 were girdled above the soil

line after 5 wk, compared with 11 of 40 seedlings from unselected tree 9. Average extent of root lesions was 4.7 cm below the soil line for tree 1 seedlings, compared with 1.7 cm for tree 9 seedlings (Table 1). The difference in root rot rating between tree 1 and tree 9 seedlings was highly significant (99.5%, *t* test). *P. lateralis* was recovered from 18% of the seedlings from tree 1 and 55% of those from tree 9.

DISCUSSION
It is evident from the field isolations and from the resistance tests that *P. lateralis* is the primary pathogen of Port-Orford-cedar in the Northwest. *P. cinnamomi*, although present in some ornamental settings and associated with dead and dying trees, was isolated only from soil, and never from forested areas in the native Port-Orford-cedar range. Thirty-five years ago, *P. cinnamomi* commonly killed Port-Orford-cedar (15); apparently, trees were infected in the nurseries before being transplanted to ornamental and hedgerow situations. This fungus species remains an important pathogen of other nursery-grown woody ornamentals in the region, but with the demise of the ornamental cedar industry (as a result of *P. lateralis*), the source of inoculum was removed. *P. cinnamomi* is unable to maintain itself during the dry Northwest summers without irrigation (11) and is not considered a threat on forested sites. *Phytophthora* species that attack other conifers in the Northwest have not been found on Port-Orford-cedar in the field and showed little or no pathogenicity to Port-Orford-cedar in inoculation tests. Therefore, we conclude that resistance screening programs can safely focus on *P. lateralis*.

P. lateralis cannot be effectively

controlled in ornamentals, and disease management in the forest is largely confined to limiting further spread into uninfested stands. Resistant Port-Orford-cedar, if available, would find immediate use. Field observations of trees surviving in the midst of dead neighbors, and the results of the limited resistance testing reported here, suggest that the population of Port-Orford-cedar varies in its susceptibility to the pathogen. In the few trees examined in detail to date, resistance is expressed as a slowing of the rate of advance of the fungus in diseased tissue, not as immunity. Screening to identify such slow-rotting individuals will require controlled, realistic inoculum levels and methods that allow measurement of the rate of disease development in individual trees.

Wound inoculation with *P. lateralis* mycelium allows the rate of lesion development to be measured directly in stem tissue of seedlings, cuttings, or branches of larger trees. Despite the artificiality of the system, results agreed well with the more natural soil and zoospore inoculations. Similar positive correlation between stem and root inoculation has been reported for *P. cactorum* and apple (13,16), *P. cinnamomi* and avocado (2) and *Eucalyptus* (14), and *P. megasperma* and soybean (6). Wound inoculation does bypass the initial stages of host-parasite recognition and infection, and resistance expressed at this point would be missed.

Wound inoculation of detached branches is particularly convenient when screening wild populations of a forest tree such as Port-Orford-cedar. Mature trees can be nondestructively tested without the problems associated with and time required for collection and

Table 1. Disease severity after inoculation with *Phytophthora* mycelium-infested soil or zoospores on excised branches, rooted cuttings, and seedlings of Port-Orford-cedars selected and not selected for resistance to *P. lateralis*

| | Stem wounding | | | Soil inoculation, cuttings | | Zoospore immersion | | | | Zoospore spray, seedlings | |
| | Cuttings | Seedlings | Branches | | | Cuttings | | Seedlings | | | |
Tree no.	Lesion length(cm)	Lesion length(cm)	Lesion length(cm)	Lesion extent[w] (cm)	Root rating[x]	Lesion extent(cm)	Root rating	Lesion height(cm)	Root rating	Lesion extent(cm)	Root rating
Selected											
1	2.7 a	5.8 a	0.4 a	−4.4 ab	1.2 a	−5.2 abc	1.2 ab	−4.6 a	1.3 a	−4.7 a[y]	1.5 a
2	3.9 a	···	0.9 a	···	···	−2.6 abcd	2.3 b	−4.2 a	1.0 a	···	···
3	···[z]	···	0.4 a	···	···	−6.9 ab	1.9 ab	···	···	···	···
4	3.5 a	···	0.5 a	−8.6 a	1.1 a	−6.9 ab	1.0 ab	···	···	···	···
Mean	3.4	···	0.6	−6.5	1.2	−5.4	1.6	−4.4	1.2	···	···
Unselected											
9	···	8.7 b	5.4 b	···	···	···	···	+1.7 a	4.5 b	−1.7 b	3.1 b
10	8.1 c	···	7.1 b	−3.3 b	3.3 b	···	···	···	···	···	···
CB11	9.7 d	···	···	−1.8 b	2.9 b	−2.2 bcde	2.4 b	···	···	···	···
CB12	5.8 b	···	···	−1.1 b	3.6 b	−0.2 cde	4.5 c	···	···	···	···
CB13	···	···	···	−4.1 ab	2.9 b	+1.8 de	4.4 c	···	···	···	···
CB14	7.4 c	···	···	−0.9 b	4.1 b	+2.8 e	5.0 c	···	···	···	···
Mean	7.8	···	6.3	−2.2	3.4	+0.6	4.1	···	···	···	···
Check	···	0.0 c	0.0 a	−5.5 ab	1.1 a	−7.8 a	0.3 a	−17 b	0.2	···	···

[w] Distance of the advancing root lesion margin below (−) or above (+) the soil line.
[x] On a scale of 0–5, where 0 = 0–10%, 1 = 11–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of roots rotted and 5 = seedling dead.
[y] Numbers in a column followed by the same letter are not significantly different (*P* = 0.05).
[z] Not measured.

propagation from cuttings or seed. Rooting cuttings from older trees is inconsistent, and the root systems that develop are variable and difficult to work with. Branches can be wounded and inoculated at any time of the year if temperatures are maintained below 25 C. More intensive resistance evaluations can then be focused on the trees that show promise in this first test. In situations where mass screening of populations of rooted cuttings or seedlings is necessary, spray inoculation with zoospore suspensions has advantages over other techniques. Many trees can be treated quickly with relatively small volumes of inoculum.

Work to date has demonstrated practical screening methodologies and revealed several promising individual trees. The resistance of these individuals must be evaluated under field conditions while the screening program is extended to more candidate trees.

LITERATURE CITED

1. Atkinson, R. G. 1965. *Phytophthora* species inciting root rot of *Chamaecyparis lawsoniana* and other ornamentals in coastal British Columbia. Can. J. Bot. 43:1471-1475.
2. Dolan, T. E., and Coffey, M. D. 1986. Laboratory screening technique for assessing resistance of four avocado rootstocks to *Phytophthora cinnamomi*. Plant Dis. 70:115-118.
3. Hamm, P. B., and Hansen, E. M. 1982. Pathogenicity of *Phytophthora* spp. to Northwest conifers. Eur. J. For. Pathol. 12:167-174.
4. Hamm, P. B., and Hansen, E. M. 1984. Improved method for isolating *Phytophthora lateralis* from soil. Plant Dis. 68:517-519.
5. Hamm, P. B., and Hansen, E. M. 1987. Identification of *Phytophthora* spp. known to attack conifers in the Pacific Northwest. Northwest Sci. 61:103-109.
6. Keeling, B. L. 1976. A comparison of methods used to test soybeans for resistance to *Phytophthora megasperma* var. *sojae*. Plant Dis. Rep. 60:800-802.
7. Kliejunas, J. T., and Adams, D. H. 1981. Phytophthora root rot of Port-Orford-cedar in California. Plant Dis. 65:446-447.
8. Linderman, R. G., and Zeitoun, F. 1977. *Phytophthora cinnamomi* causing root rot and wilt of nursery-grown native western azalea and salal. Plant Dis. Rep. 61:1045-1048.
9. Ostrofsky, W. D., Pratt, R. G., and Roth, L. F. 1977. Detection of *Phytophthora lateralis* in soil organic matter and factors that affect its survival. Phytopathology 67:79-84.
10. Riker, A. J., and Riker, R. S. 1936. Introduction to Research on Plant Diseases. Swift, St. Louis, MO. 117 pp.
11. Roth, L. F., and Kuhlman, E. G. 1966. *Phytophthora cinnamomi*, an unlikely threat to Douglas-fir forestry. For. Sci. 12:147-159.
12. Roth, L. F., Trione, E. J., and Ruhmann, W. H. 1957. *Phytophthora* induced root rot of native Port-Orford-cedar. J. For. 55:294-298.
13. Sewell, G. W. F., and Wilson, J. F. 1959. Resistance trials of some apple rootstock varieties to *Phytophthora cactorum* (L. and C.) Schroet. J. Hortic. Sci. 34:51-58.
14. Tippett, J. T., Hill, T. C., and Shearer, B. L. 1985. Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust. J. Bot. 33:409-418.
15. Torgeson, D. C., Young, R. A., and Milbrath, J. A. 1951. Phytophthora root rot diseases of Lawson cypress and other ornamentals. Oreg. Agric. Exp. Stn. Bull. 537.
16. Utkhede, R. S. 1986. In vitro screening of the world apple germplasm collection for resistance to *Phytophthora cactorum* crown rot. Sci. Hortic. 29:205-210.
17. Zobel, D. B., Roth, L. F., and Hawk, G. M. 1985. Ecology, pathology, and management of Port-Orford-cedar (*Chamaecyparis lawsoniana*). U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. PNW-184. 161 pp.