Histopathology of Infection and Colonization of Susceptible and Resistant Port-Orford-Cedar by *Phytophthora lateralis*

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Accepted for publication 10 January 2007.

ABSTRACT

Oh, E., and Hansen, E. M. 2007. Histopathology of infection and colonization of susceptible and resistant Port-Orford-cedar by *Phytophthora lateralis*. *Phytopathology* 97:684-693.

Port-Orford-cedar (POC) root disease, caused by *Phytophthora lateralis*, continues to kill POC in landscape plantings and natural forests in western North America. POC trees resistant to *P. lateralis* have been identified and propagated. Cytological observations of *P. lateralis* in susceptible and resistant roots and stems were made with light and transmission electron microscopy to identify resistance mechanisms. No differences in infection pathway and initial colonization were observed between susceptible and resistant roots, although there were differences in the rate and extent of development. Germ tubes formed appressoria, and penetration hyphae grew either between or directly through epidermal cell walls; inter- and intracellular hyphae colonized the root cortex. In susceptible roots, hyphae penetrated into the vascular system within 48 h

of inoculation. In contrast, hyphae in roots of resistant seedlings grew more slowly in cortical cells and were not observed to penetrate to the vascular tissues. In resistant roots, infection was marked by general thickening of cortical cell walls, wall appositions around penetrating hyphae, collapse of cortical cells, and accumulation of osmophilic granules around hyphae. In susceptible stems, hyphae grew inter- and intracellularly in all cells of the secondary phloem except fiber cells, but were concentrated in sieve and parenchyma cells in the functional phloem. The pattern of penetration and colonization of hyphae was similar in the resistant stems, except that hyphae were found in the fiber cells of the xylem. In resistant stems, there were fewer hyphae in the functional phloem, and cytological changes such as damaged nuclei and disintegrated cytoplasm were evident. Structural changes in resistant stems included collapsed cells, wall thickening, secretory bodies, apposition of electron dense materials, and crystals in cell walls.

Species of *Phytophthora* are important pathogens of forest trees around the world, threatening both ecological and economic values. Resistance selection and breeding programs are underway for chestnut in Europe (12) and radiata pine and jarrah eucalyptus in Australia (4,10,15) susceptible to *Phytophthora cinnamomi*. In the western United States, Port-Orford-cedar (POC) (*Chamaecyparis lawsoniana* (A. Murray Parl.) trees resistant to *P. lateralis* Tucker & Milbrath have been identified, and a resistance breeding program is central to POC root disease management on public and private lands (8,17). In the present work, we compare pathogen colonization and host responses at the cellular level in roots and stems of resistant and susceptible POC trees.

POC is an important native forest tree with a limited natural range in southwestern Oregon and northern California. It is valued for the decay resistance and milling properties of its wood, and for its ecological roles, especially in riparian areas and on ultramafic soils (34). POC also was valuable as a landscape tree, with many named cultivars. The introduction of *P. lateralis* ended the horticultural trade in POC and threatens the ecological and economic viability of the species in forest settings (1,34).

POC root disease was first reported in horticultural nurseries in the Seattle, WA area in the 1920s, and in natural coniferous forests in Oregon in the 1950s (8,20,26,27). Trees of all ages are killed within a few months or years of infection by this invasive pathogen. The disease continues to spread and is now found throughout the natural range of POC, as well as in landscape plantings along the West Coast of the United States and Canada (8).

Heritable resistance in POC to *P. lateralis* was first described in 1989 (9), and a cooperative program with the United States Department of Agriculture (USDA) Forest Service, United States Department of the Interior Bureau of Land Management, and Oregon State University to identify, propagate, and breed for resistance has been operating since that time. First-generation resistant POC seedlings from a containerized seed orchard are now available to forest managers (13,14,16), and resistant trees are beginning to reenter the horticultural industry.

Between 50 and 75% of the seedlings from selected resistant parents survive short-term greenhouse inoculation tests in which only ~5% of wild-type seedlings survive (16,17). Comparable survival is shown in field trials. Seedlings and rooted cuttings from the earliest selections continue to grow vigorously on infested sites after 20 years of exposure to the pathogen. Controlled crosses between selected parents suggest that several genetic mechanisms are involved. Resistance in a few trees is controlled by one or a few major genes, whereas other trees express a more complex quantitative resistance (17).

Tucker and Milbrath (29) first described *P. lateralis* and the symptoms and development of POC root disease. The pathogen is homothallic. Although oospores are seldom seen in culture, chlamydospores and sporangia are readily formed. Infection of POC usually is initiated by motile zoospores attracted to fine lateral roots in saturated soils (8). The pathogen colonizes the live inner bark on larger roots, killing the phloem of roots and, ultimately, the base of the stem (20,27,29). Field diagnosis of POC root disease is based on the characteristic reddish-brown color of recently killed inner bark, with necrosis extending up the bole from the roots in broad streaks, and with a sharp margin between necrotic and still-living inner bark tissues. Although the general course of infection is known and the resulting necrotic symptoms are famil-

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doi:10.1094/PHYTO-97-6-0684

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iar, there has been no description of infection and pathogenesis at the histological level on either susceptible or resistant POC trees.

Histological and cytological observations of several *Phytophthora* diseases have been reported (2,6,25), including, in a few cases, comparisons between resistant and susceptible species and cultivars of a species. Infection by *P. nicotianae* and *P. palmivora* on fibrous roots of citrus species that have different host responses to infection was compared by means of light and electron microscopy (32). Similarly, histological changes in susceptible and resistant plant species inoculated with *P. cinnamomi* were examined (3). A histopathological study of soybean hypocotyls inoculated with *P. sojae* contrasted reactions of resistant and susceptible cultivars (23), and the general resistance reactions of potato cultivars at the cytological level were described (19,33).

Recently, a histological study of *P. ramorum*, the causal agent of sudden oak death and a close genetic relative of *P. lateralis*, described the colonization of *Rhododendron* tissue (18). There have been no histological descriptions of either susceptible or resistant reactions to *P. lateralis* in POC, however. In this article, we use bright-field and fluorescence light microscopy and electron microscopy to illustrate infection and colonization of POC by *P. lateralis*. Reactions of susceptible and resistant POC seedlings are compared.

MATERIALS AND METHODS

POC seedlings and cultures. Two-year-old seedlings or rooted cuttings of susceptible and resistant POC were provided by the Dorena Genetic Resource Center, USDA Forest Service in Cottage Grove, OR (Table 1). Seedlings from open-, self-, and cross-pollinated families as well as rooted cuttings from selected parent trees were included. Seedling families and parent trees had been tested previously for susceptibility to *P. lateralis* (17). Two isolates of *P. lateralis*, 368 and T4P3, obtained from Gasquet Ranger District, Six Rivers National Forest, CA in 1986 and Galice District, Siskiyou National Forest, OR in 2000, respectively, were used for root and stem inoculation. Cultures were grown on β -corn meal agar (β -CMA) (Difco Laboratories, Detroit), amended with β -sitosterol (Acros Organics, Morris Plains, NJ) at 20 ppm and stored in liquid nitrogen or water.

For zoospore production, isolates were grown for 7 days in pea broth (150 g of split pea in 1 liter of dH₂O autoclaved for 4 min and filtered) with β -sitosterol at 20 ppm added after autoclaving. The pea broth was replaced with stream water and cultures incubated an additional 2 days at 17°C to induce sporangia. Plates were chilled and returned to room temperature to trigger zoospore release. Zoospore concentration was determined with the aid of a hemacytometer.

Inoculation of roots and stems. POC seedlings and rooted cuttings were inoculated on actively growing primary roots or on 2-year-old stem tissues. The tips (2 cm) of actively growing lateral roots of susceptible and resistant cedar seedlings were removed and washed with distilled water. Excised root tips were floated for 24, 48, and 72 h on 30 ml of zoospore suspension (3.6×10^5 zoospores ml⁻¹) of isolates 368 and T4P3. Control roots were floated

on distilled water at the same time and prepared for microscopy similarly. The experiment was repeated three times, and duplicate samples were observed in each trial.

POC stems were inoculated by inserting mycelia into a small incision in the bark (17). Isolates were grown in pea broth as described above, rinsed in deionized water, and the mycelium used for inoculation. Small transverse cuts were made to the cambium and portions of mycelium placed beneath the flap of bark, then covered with wet cheese cloth and foil to prevent drying. The inoculated seedlings (three to five inoculations for each family or clone) were incubated in a greenhouse for 4 weeks. The lengths of necrotic lesions were recorded after bark was peeled off on one side of the stem, and then two 2-mm-long pieces were removed from the opposite side of the stem for sectioning. These pieces were removed at the necrotic margin (transition between green and necrotic tissues). Wounded but uninoculated POC stems were included as control. Differences in necrotic lesion length between POC families were tested by analysis of variance using Statgraphics software (Statpoint, Inc., Herndon, VA). The Scheffe multiple range test was used to compare family mean lesion lengths.

Microscopy. The samples of root and stem for light microscopy (LM) were fixed individually in 2.5% glutaraldehyde in 0.2 M sodium phosphate buffer at 4°C overnight. After washing three times for 10 min with the same buffer, dehydration was performed with a graded ethanol series (30, 50, 75, and 95 or 100%) for 1 h each, and the last step was repeated once. The samples were embedded using the Technovit 7100 embedding kit following the manufacturer's instruction (EBScience Co., Agawam, MA). The embedded samples were sectioned to 4 μ m-thick using a rotary microtome (Spencer Lens Co., Buffalo, NY).

Cellulosic cell walls stained with Calcofluor (Sigma-Aldrich, St. Louis) fluoresce bright blue or white, enhancing the contrast between host tissues and hyphae. Sections were transferred to slides in a drop of double-distilled (dd)H₂O, stained with Calcofluor 0.001% Fluorescent Brightener 28 in 0.05 M Tris-HCl (pH 8.0) (28) or in ddH₂O for 10 min, rinsed with ddH₂O, mounted with Polymount (Polysciences, Inc. Warrington, PA), and examined with epifluorescence (Zeiss Axioskop II, Carl Zeiss Inc.) using excitation filter 450-490 and emission filter 515.

Samples for transmission electron microscopy (TEM) were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer and postfixed with 2% OsO₄ for 2 h at room temperature, then embedded (22) as described above. A 2-mm-long segment of the embedded root was cut from the root tip, trimmed, and sectioned using an MT-2 ultramicrotome (Sorvall Poster-Blum; Ivan Sorvall Inc., Norwalk, CT) equipped with a diamond knife (Polysciences Inc. Niles, IL). The sections were mounted on formvar-coated copper grids and stained with lead citrate (Reynold's stain) (30) using the double lead stain technique (7). The sections on the grids were examined with TEM (Philips CM12) at 60 kV with 100- μ m objective apertures at magnifications ranging from $\times 3,800$ to $\times 22,000$. Photographic images were recorded on a 3 1/4- by 4-in. plate camera using Kodak 4489 film (Eastman Kodak Co., Rochester, NY).

TABLE 1. Reaction in resistance screening tests (17) and origin of Port Orford cedar seedlings

Sow ^a	Family/clone ^b	Expected reaction ^c	Origin
48	117499xOP	S	Gold Beach District, Siskiyou National Forest (NF), OR
...	118051xOP	S	Illinois Valley District, Siskiyou NF, OR
50	510015xOP	S/R ^d	Gasquet District, Six Rivers NF, CA
53	CF1xOP	S/R	Coos Bay, OR
16	117490xOP	R	Gold Beach District, Siskiyou NF, OR
RT1204	70037RT	S	Gold Beach District, Siskiyou NF, OR
RT1317	CF1RT	R	Coos Bay, OR
...	117490RT	R	Gold Beach District, Siskiyou NF, OR

^a Sow number is Dorena Genetic Resource Center code. ... = number not available.

^b Family by open pollination (OP) and clone by rooted cutting (RT).

^c S = susceptible and R = resistant based on root dip inoculation (19).

^d Hypothesized to segregate susceptible and resistant offspring at 3:1 or 1:1 ratios.

RESULTS

Penetration and colonization of roots. Transverse sections of healthy, uninfected roots were composed of layers of epidermal cells and cortical cells surrounding the vascular core. The outer layer of epidermis had irregular cell shape, size, and arrangement, especially near the root cap. Also, the outer layers sloughed off easily. Cells in the cortex were uniform in shape and size (Fig. 1A). Cell walls of epidermal and some exodermal cells fluoresced more brightly than cortical cells after staining with Calcofluor. The vascular cylinder was composed of xylem and phloem surrounded by a layer of pericycle and endodermis (Fig. 1B).

Excised, nonsuberized root tips of susceptible and resistant POC seedlings were exposed to zoospores of *P. lateral* for various times or left uninoculated, stained with Calcofluor, and observed with fluorescence microscopy. No structures resembling zoospores, cysts, or hyphae were observed in or on uninoculated roots. However, on inoculated roots, *P. lateral* cysts were observed to germinate, penetrate, and colonize both susceptible and resistant families, although each step appeared to be less frequent and slower in resistant than in susceptible roots. The germ tubes sometimes were divided by a septum and often formed an appressorium before penetration of the root. Initial penetration usually occurred between the anticlinal walls of the epidermal cells (Fig. 1C).

Fig. 1. Fluorescence micrographs of transverse sections of Port-Orford-cedar roots exposed to zoospores of *Phytophthora lateral* for 24 h and stained with 0.001% Calcofluor (Sigma-Aldrich, St. Louis). **A** and **B**, Uninoculated 118051xOP and 510015xOP. **C–F**, 118051xOP susceptible roots; **G–H**, 510015xOP resistant roots. **C**, Penetration of hyphae between cells (arrow). **D**, Direct penetration of hyphae into a cell (arrow). **E**, Branching hypha at epidermis–cortex interface (arrow). **F**, Colonization in cortex. **G** and **H**, Ungerminated and germinated cysts (arrows). CO = cortex, EN = endodermis, EP = epidermis, PE = pericycle, PH = phloem, ST = stele (vascular cylinder), and X = xylem.

Direct penetration through an epidermal cell wall also was observed (Fig. 1D). After penetration through the root epidermis, the hyphae were delimited by septa and continued to elongate until reaching cortical cells (Fig. 1C). Hyphae grew through the epidermis to the open space between the separated outer layer of epidermis and the first layer (hypodermis) of cortex. This region often fluoresced brightly (Fig. 1E).

The main penetrating hyphae became swollen and extended their branches to neighboring intact cortical cells (Fig. 1E and F). Many-branched hyphae elongated and colonized the parenchyma cells of the cortex. The branched hyphae grew intercellularly and intracellularly in the cortex (Fig. 1F). Fewer hyphae were observed in roots of susceptible families 117499×OP and 70037RT than in susceptible family 118051×OP; however, there were no apparent differences among the susceptible families examined in the pattern or timing of germination, penetration, and colonization.

In susceptible roots incubated with zoospores for 24 h, hyphae were observed throughout the cortex and up to the boundary of the vascular tissues. After 48 h, roots contained more hyphae in the cortical tissue, and hyphae were seen in the vascular system as well. After 72 h, root segments appeared much as they had at 48 h.

Roots of resistant trees (510015×OP, 117490RT, and CF1RT), inoculated and stained together with the susceptible roots, showed fewer cysts on the root surfaces and fewer examples of germination and penetration. Most cysts were not germinated (Fig. 1G) but, when germinated, penetration occurred as in susceptible roots, with both direct invasion into the epidermal cells and penetration between epidermal cells observed (Fig. 1H). After 24 h, hyphae in resistant roots had not penetrated as deeply in the cortex as in susceptible roots. More hyphae were observed in the outer two layers of cortical cells in 117490RT and CF1RT than in 510015×OP, but otherwise there were no apparent differences among the resistant clones and families. Even after 72 h, no hyphae were observed in the vascular system of resistant roots. There were no asexual structures (sporangia or chlamydospores) or sexual structures (oogonia and oospores) observed in the roots of either susceptible or resistant POC seedlings in this study.

Ultrathin sections of uninoculated roots were examined by TEM as a basis for comparison with infected roots, concentrating on epidermal and cortical cells near the surface of the root. Usually, the dead epidermal cells did not contain cytoplasm (Fig. 2A). The cortical cells beneath epidermal cells were either empty or full of cytoplasmic material. Germinated cysts, appressoria, and penetration hyphae were observed on susceptible roots (Fig. 2B–F). Numerous intra- and intercellular hyphae were present in cortical cells as well as epidermal cells after 12 h of incubation (Fig. 2E and F). After 24 h of incubation, electron-dense materials were observed near hyphal cell walls in a few cases.

Infected roots of resistant tree 117490 presented a different image. A few cysts were present on root surfaces, but they were seldom germinated. One germinated cyst produced an appressorium or swollen hypha beneath epidermal cells; however, its cytoplasm was electron dense, with evident disruption near a thickened host cortical wall, in contrast to healthy-appearing penetration hyphae in susceptible roots. Host cell-wall modifications, including increased cortical wall thickness, commonly were observed in the resistant roots. An ungerminated cyst was observed at 9 h after inoculation, and wall appositions were present in nearby cortical cells (Fig. 2K and L). After 5 h of incubation, electron-dense materials were seen in cortical cells beneath a layer of epidermal cells without attached cysts (Fig. 2H). The walls of some cortical cells, deposited with electron-dense materials, appeared collapsed (Fig. 2I). Cell wall thickening was observed in sections from infected resistant roots whether cysts were evident in the section or not. After 7 h of incubation, intracellular hyphae were seen in cortical cells (Fig. 2J). A collar-like wall apposition that included osmiophilic granules between cell wall and membrane was observed where a hypha was penetrating a host cell wall. The intracellular

hyphae were surrounded by numerous osmiophilic granules. Wall thickenings, as well as electron-dense materials, also were present along the walls (Fig. 2L). Infected roots of resistant tree CF1RT were similar to 117490, except that no collapsed cells containing electron-dense materials were observed in CF1 (not shown).

Colonization of stems. Wound inoculation initiated necrotic lesions in stems of both susceptible and resistant POC trees. When the outer bark was scraped off, a distinct margin between healthy and necrotic tissues was evident (Fig. 3A and B). Lesions were shorter in resistant trees than in susceptible trees; control inoculations produced no necrotic lesions. In addition, in resistant trees, resin was exuded through the bark near necrotic areas (Fig. 3C). Differences in necrotic lesion length were significant between families (F ratio = 7.33, P = 0.0004). Seedlings of families 117499×OP and CF1×OP and rooted cuttings of 70037RT had longer necrotic lesions (family average) than other families or clones. Seedlings of family 510015×OP were intermediate in average lesion length, and 117490×OP seedlings and rooted cuttings of CF1RT and 117490RT had the shortest lesions of the trees tested (Fig. 4).

All cells appeared healthy in cross sections of uninoculated stems stained with Calcofluor, and no hyphae were observed. Secondary xylem, vascular cambium, phloem, and a developing peridermal tissue (Fig. 5A and B) were distinguished. The peridermal tissue included primary phloem, cork cells, epidermis, and cuticle. The secondary phloem was composed of functional phloem, ray parenchyma, and the nonfunctional phloem cells of the cortex (Fig. 5A). The functional phloem included phloem mother cells, followed by fiber cells surrounding two layers of sieve cells, enclosing the parenchyma cells (Fig. 5B).

Inoculated POC trees were sectioned through the margin of the necrotic lesion. In susceptible trees, hyphae were seen in the cambium, in the sieve and parenchyma cells of the functional phloem, and in cortical cells, but fiber cells were not colonized (Fig. 5C and D). Hyphae were intercellular in the cambial layer of all susceptible stems (Fig. 5C). Many intercellular and intracellular hyphae were observed in phloem sieve cells and especially in parenchyma cells of 117499×OP (Fig. 5C) and 118051×OP (Fig. 5D). 70037RT was similar, except that fewer hyphae were observed than in 117499×OP and 118051×OP. A few hyphae also were present in cortical cells of 70037RT. Some cortical cells of this clone did not have any hyphae and did not fluoresce, in contrast to the brightly fluorescent cells of uninoculated stems.

In all resistant stems, colonization by the hyphae was highly restricted (Fig. 5E). Most hyphae were observed in cambial cells and the outermost layers of sieve and parenchyma cells (Fig. 5E). Some cells were nonfluorescent with or without visible hyphae and, in many cases, cells in the cambium and phloem were collapsed or disorganized without visible hyphae (Fig. 5F). Nonfluorescent cells were seen in all sections of the necrotic margin of 117490×OP and 117490RT. Intercellular hyphae were present between cambium cells and between sieve and parenchyma cells in phloem on CF1×OP (Fig. 5G). Interestingly, hyphae were observed in xylem cells of CF1×OP and CF1RT stems, but not in other trees (Fig. 5H). Often, more hyphae were observed in the xylem than in the phloem of CF1.

Ultrathin sections of an uninoculated stem of CF1RT and 510015×OP were examined by TEM to establish normal structure of POC stem cells (Fig. 6A). Parenchyma cells in the cortex were intact and some crystals were visible between radial cell walls of all cell types. Parenchyma cells contained nuclei, starch grains, and lipid-like bodies and appeared healthy (Fig. 6B). In inoculated stems of susceptible 70037RT, intracellular hyphae were observed in sieve (Fig. 6C) and parenchyma cells. Electron-dense materials were seen near starch plasmids in parenchyma cells. A chlamydospore was observed between cells. Electron-dense materials such as lipid bodies were present along cell walls (Fig. 6D).

In sections from the necrotic margin of resistant 117490RT stems, starch bodies and lipid-like bodies in parenchyma cells ap-

Fig. 2. Electron micrographs of ultrathin sections of susceptible (70037RT) and resistant (117490RT) roots of Port-Orford-cedar inoculated with *Phytophthora lateralis*. **A**, Epidermal cells (EP) and external zone (EX) of a control root of 510015×OP. **B–F**, Susceptible roots and **G–L**, resistant roots. **B**, Germinated cysts (CY) on an epidermal cell at 5 h after inoculation. **C**, Ungerminated cysts (CY) on an epidermal cell at 5 h after inoculation. **D**, Cyst with an appressorium (AP), and swollen structure penetrating into an epidermal cell after 9 h. **E**, Intracellular (IA) hyphae in an epidermal cell after 12 h. **F**, Abundant intercellular (IR) and intracellular hyphae in cortical cells after 12 h. **G**, Germinated cyst with an appressorium after 1 h. **H**, Wall apposition beneath the epidermis after 5 h. **I**, Wall apposition and collapsed cell walls after 5 h. **J**, Simultaneous wall modification (arrow) and penetration of intracellular hypha in cortical cells after 7 h. **K**, Ungerminated cyst and wall apposition (arrow) by electron-dense materials beneath epidermis after 9 h. and **L**, Cell-wall (CW) thickening behind the epidermis after 9 h. CC = cortical cell.

peared different than in control and inoculated susceptible stems (Fig. 6E). The starch bodies were surrounded by osmiophilic granules. Resin was observed on cell walls, as in uninoculated resistant stems. The lipid-like bodies were not solid, but full of granular materials. An intracellular hypha with a broken cell wall was observed in one parenchyma cell. The hypha was surrounded by numerous secretory bodies in the host cytoplasm, and the bodies were contiguous with the sheath-like layer. Cell walls in resistant stems were thicker than the walls in control and inoculated susceptible stem sections (Fig. 6F). The secretory bodies were covered with a kind of sheath that was associated with the cell wall. In addition, an intracellular hypha in a parenchyma cell was observed penetrating to the adjacent cell. The host cell wall was thickened at the point where the hypha penetrated (Fig. 6G). Electron-dense material was observed between the wall and the hypha, appearing to prevent attachment of the hypha to the host cell wall (Fig. 6H and I). In general, intracellular hyphae in resistant stems looked more abnormal than in the susceptible stem. Additional observations were made on intracellular hyphae surrounded by electron-dense materials in parenchyma cells. In CF1RT, more resin crystals were found on the radial and occasionally on the tangential cell walls in the secondary phloem. Also, cytoplasm in most parenchyma cells was gone (Fig. 6J and K). Some starch bodies still remained in the parenchyma cells but nuclei were damaged (Fig. 6K), with disrupted membranes and nucleoplasm. No hyphae were observed in CF1, but a chlamydospore was present between cells (Fig. 6L).

DISCUSSION

P. lateral zoospore germination and penetration were similar on primary roots of susceptible and resistant seedlings, although the frequency of penetration events appeared to be lower in resistant trees. Like other *Phytophthora* spp. (2,6,19,23,32), *P. lateral* commonly formed appressoria on root surfaces and penetrated between anticlinal epidermal cell walls, with occasional direct penetration of epidermal cells.

At the ultrastructural level, cell-wall thickening, even in cells distant from visible hyphae of *P. lateral*, commonly was observed in inoculated resistant roots but not in susceptible roots. Host cell-wall appositions at points of hyphal penetration were present in resistant roots. In addition, cortical cell walls below the epidermis in inoculated resistant roots were crumpled and cell contents disrupted. General host cell-wall thickening and localized wall appositions also have been observed in resistant

reactions in other host–pathogen combinations, including *P. sojae*–soybean, *P. cambivora*–chestnut, and *P. infestans*–potato (5,11,24,31,33).

POC responses to infection in the secondary tissues of inoculated stems were similar to those observed in roots. Phloem cells (except phloem fiber cells) were colonized both inter- and intracellularly. Haustoria were not observed. No hyphae were seen in the bark. Fewer hyphae were evident in resistant stems of all families and, as in roots, *P. lateral* colonized the secondary xylem in the stem of CF1 but not 117490.

Ultrastructural and cytological changes, including cell-wall thickening, also were observed in resistant stems inoculated with *P. lateral*. Numerous secretory bodies were observed in the host cytoplasm in cells with adjacent hyphae and the hyphae and adjacent host cell walls were surrounded by a sheath-like layer. Per-

Fig. 4. Mean length of necrotic lesions on stems of Port-Orford-cedar seedlings inoculated with *Phytophthora lateral* mycelium and incubated for 4 weeks. Bars with the same letter are not significantly different ($P = 0.05$, Scheffe multiple range test).

Fig. 3. Port-Orford-cedar seedlings 4 weeks after stem wound inoculation with mycelium of *Phytophthora lateral*. **A**, Susceptible (a) 117499xOP and resistant (b) 510015xOP and (c) 117490xOP. **B**, Distinct necrotic margin on 117490xOP (arrow). **C**, Resin flow on inoculated 117490xOP.

haps these cell modifications formed a barrier, preventing extension of hyphae and restricting colonization of host tissue (6,11, 24). Unidentified resin accumulated more abundantly in inoculated resistant stems than in healthy stems or inoculated susceptible stems, suggesting that the deposition of crystals in the resis-

tant clones of POC is another defense response against *P. lateralis*.

In summary, *P. lateralis* infection of POC trees follows similar pathways to those described for *Phytophthora* spp. on other hosts. Roots of susceptible trees are colonized quickly and without evi-

Fig. 5. Fluorescent micrographs of transverse sections of susceptible and resistant stems of Port-Orford-cedar stained with 0.001% Calcofluor (Sigma-Aldrich, St. Louis). **A**, 118051×OP control, secondary phloem. **B**, 118051×OP control, functional phloem. **C**, 117499×OP, intercellular hyphae (IR) in the vascular cambium and in phloem mother cells in 117499×OP. **D**, 118051×OP, intercellular hyphae in phloem, intracellular hyphae (IA) in sieve and parenchyma cells. **E**, 117490×OP, fewer hyphae in phloem. **F**, 117490RT, collapsed cells. **G**, CF1×OP, inter- and intracellular hyphae in cambial, sieve, and parenchyma cells of phloem as well as intracellular hyphae in xylem. **H**, CF1RT, colonized hyphae in xylem. CO = cortex (nonfunctional phloem), FP = functional phloem, PC = parenchyma cell (tannin cell), PMC = phloem mother cell, PP = primary phloem, RP = ray parenchyma (living), SC = sieve cell; SP = secondary phloem (inner bark), SX = secondary xylem, and VC = vascular cambium.

Fig. 6. Electron micrograph of ultrathin sections of susceptible and resistant Port-Orford-cedar stems. **A**, CF1RT control, intact functional phloem. **B**, 510015×OP control, intact parenchyma cell. **C**, 70037RT, an intracellular hypha (IA) in a sieve cell. **D**, 70037RT, a chlamydospore (CL) between cells. **E**, 117490RT, intracellular crystals (CT) in a parenchyma cell containing starches and lipid-like bodies. **F–I**, 117490RT, structural changes of parenchyma cells by electron-dense materials, including secretory bodies associated with intra- and intercellular hyphae. **J–L**, CF1RT, cytological changes in parenchyma cells and a chlamydospore. CW = cell wall of host, FC = fiber cell, LB = lipidlike bodies, N = nucleus, PC = parenchyma cell, SB = secretory bodies, SC = sieve cell, and ST = starch.

dent host response. Colonization of resistant trees, in contrast, is slower and less complete, at least in the first days followed here. Resistant trees exhibit a variety of directed responses to the pathogen in primary roots as well as in woody stems, including general wall thickening, cell collapse, and deposition of electron-dense materials around hyphae. These reactions are consistent with a hypersensitive host response.

Selecting and breeding POC for resistance to *P. lateral*is has become an integral part of the effort to restore and maintain the ecological and economic viability of POC in forests. Availability of disease-resistant trees also might restore this species to its former horticultural importance. A very few wild trees survive the pathogen even in areas of severe disease pressure and have been shown to have heritable resistance (9,14). Hundreds of POC trees have been identified in the forest, propagated, and tested to demonstrate heritable resistance (17). Three of the early selections (117490, CF1, and 510015) were included in this study. Tree 117490, in particular, may have a major gene for resistance. There are indications of quantitative resistance in other trees (17,21).

Resistance is expressed most importantly as improved survival rates of seedling families from resistant parents and, in some trees, by development of short, sunken, resinous lesions following wound inoculation (17). Ideally, trees expressing different modes of resistance controlled by different genes will be found in trees representing the full genetic diversity of the POC population. Breeding zones based on growth differences in common garden studies have been established across the full natural range of POC, and selection for resistance is proceeding independently in each natural population. Separate seed orchards are being established for each breeding zone in order to preserve the full genetic diversity of the species. Still, the long-term durability of resistance in POC to *P. lateral*is will depend on the diversity of resistance genes present in the breeding populations as well as the diversity of virulence genes in the pathogen population. To date there is no evidence for changes in virulence in the pathogen population in the areas where resistant trees have been grown the longest (13,17).

Two resistant parent trees, CF1 and 117490 (coincidentally from different breeding zones), are best characterized. Mendelian segregation ratios for survival among progeny from controlled crosses with susceptible parents suggest major resistance genes at one or a few loci (17). The reaction of CF1 to infection differs in some ways from 117490, with at least limited xylem colonization and indications of differences in zoospore attraction to roots (data not shown). The histological evidence suggests that resistance is expressed differently in these two trees. Other trees appear to have quantitatively inherited resistance, although its expression is still poorly defined. Future work on quantitative resistance mechanisms will require plant materials that are better characterized genetically. Tree-breeding experiments are underway, but breeding in trees, even the relatively malleable POC, is a slow process.

ACKNOWLEDGMENTS

This work was supported by the United States Forest Service and Bureau of Land Management. We thank R. A. Snieszko and the staff at the Dorena Genetic Resource Center, USDA Forest Service, Cottage Grove, OR, for providing POC seedlings; and J. Stone for important assistance with microscopic technique and the interpretation of results.

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