# Foliar Blight and Root Rot of Container-Grown Giant Redwood Caused by *Phytophthora citrophthora*

C. M. SANDLIN, Graduate Research Assistant, and D. M. FERRIN, Assistant Professor, Department of Plant Pathology, University of California, Riverside 92521

**ABSTRACT**

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*Phytophthora citrophthora* was isolated from the blighted foliage of container-grown giant redwoods (*Sequoiadendron giganteum*) grown under overhead irrigation at a nursery in southern California. The isolate caused both foliar blight and root rot when inoculated onto greenhouse-grown redwood seedlings. Scanning electron microscopy of the foliar blight stage showed that zoospores aggregate at and infect via stomata. Inoculation of the roots of 1-mo-old seedlings resulted in a significant reduction in the mean dry weight of the foliage after 3 mo (51.1 and 33.6 g for the uninoculated and inoculated treatments, respectively) and a change in the morphology and distribution of the roots within the pots. Isolates of *P. citrophthora* from *Penstemon, Ceanothus*, and *Citrus* and an isolate of *P. parasitica* from *Pinus pinea* also caused foliar blight.

During the fall of 1990, a foliar blight of container-grown giant redwood (*Sequoiadendron giganteum* (Lindl.) Buchholz) developed at an ornamental nursery in southern California. Large branches, stems, and twigs were affected. Blue-black lesions typically delineated the lower margins of the dieback. Tissue below these lesions was healthy, whereas the foliage above the lesions was dead and brown or in the process of dying. *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian was isolated from the lesions, and its pathogenicity on giant redwood foliage was demonstrated (9).

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Most commonly found as root pathogens, species of *Phytophthora* often cause problems in ornamental and forest nurseries (4). The warm, humid conditions maintained in greenhouses can lead to foliar diseases, as can the use of overhead irrigation. McCain and Scharpf (5) reported a shoot blight and canker disease of another conifer, *Abies* spp., caused by *Phytophthora citricola* Sawada; the disease was associated with the use of sprinkler irrigation at a Christmas tree plantation.

The blighted redwood trees examined in these studies had been maintained under overhead irrigation, and the cool, humid climate of coastal southern California may have exacerbated the situation. Foliar symptom development halted when plants were moved from the nursery to a greenhouse where they were hand-watered rather than irrigated from above. The plants remained in poor health, however, with little growth of new foliage.

The studies described here were conducted to further our understanding of the biology of the disease in a way that might aid in disease control. Specifically, the studies were designed to determine how infection occurs, the importance of moisture in disease development, whether the ability to cause disease on redwood is unique to the isolates of *P. citrophthora* from *S. giganteum*, and if the pathogen can infect roots as well as foliage.

## MATERIALS AND METHODS

**Microscopic observation of zoospore encystment and infection of plant tissue.** Young shoots (about 10 cm long) were cut from 6-mo-old *S. giganteum* seedlings and floated for 15 min in a suspension of $1 \times 10^5$ zoospores per milliliter. Zoospores were produced from sporangia on mycelia grown at 20 C in autoclaved V8 juice-$CaCO_3$ broth in 100 × 15-mm plastic petri dishes. The broth was made by centrifuging a mixture containing 5 g of $CaCO_3$ and 354 ml of V8 juice for 20 min at 4,000 rpm and diluting the resulting supernatant with deionized water (1:4, v/v, supernatant to water). Three days after inoculation of the broth, the resulting mycelial mats were teased into several small pieces and allowed to grow for an additional day. The production of sporangia was induced by rinsing the 4-day-old mats five times with sterile deionized water and incubating them overnight in soil extract (6 ml of extract per petri dish) under fluorescent light. The soil extract was made by autoclaving 1 g of air-dried soil (garden loam) in 500 ml of water. The extract was decanted before use. Zoospore release was induced by rinsing the mycelial mats and sporangia with chilled (4 C) soil extract.

Several of the shoot pieces were fixed immediately after inoculation, and the remaining samples were incubated at 20 C on wet pieces of filter paper in sealed glass petri dishes in the dark. Samples of the incubated shoot pieces were subsequently fixed 1, 3, 6, and 9 hr after inoculation.

Tissue samples were fixed in a 2.5% solution of glutaraldehyde (by volume) in 0.1 M phosphate buffer (pH 7.2), rinsed five times in 0.1 M phosphate buffer (pH 7.2), and then postfixed in a solution of $OsO_4$ (10 g of $OsO_4$ per liter) in 0.1 M phosphate buffer (pH 7.2). Samples were dehydrated by transfer through an ethanol series (30, 50, 70, 95, and 100%), and dried in an Autosamdri-810 critical point dryer (Tousimis Research Corporation, Rockville, MD). They were then coated with gold on a Hummer V sputter coater (Technics, Alexandria, VA) and viewed at 15 kV on a JSM 35 C scanning electron microscope (JEOL, Tokyo, Japan).

**The role of free moisture in disease development.** Plants used for foliar inoculation studies were grown from seed in 1-L plastic pots filled with UC-4 mix, (a steam-pasteurized potting mix composed of 0.382 $m^3$ of sand, 0.382 $m^3$ of bark, 3,401.9 g of dolomite, 1,134.0 g of limestone flour, 1,134.0 g of treble superphosphate, 56.7 g of $KNO_3$, 56.7 g of KCl, 62 g of $FeSO_4$, 22.6 g of $ZnSO_4$, 43 g of $CuSO_4$, 17 g of $MnSO_4$, and 34 g of $MgSO_4$). The seedlings were 6–8 mo old when inoculated.

To investigate the role of moisture on the development of disease, plants were subjected to different periods of free moisture and high humidity after inoculation. Six plants were first misted with water and placed in the dark for 1 hr before inoculation. Five of the plants were then inoculated by pipetting 25 ml of a zoospore suspension ($1 \times 10^5$ zoospores per milliliter) onto the foliage of each plant. The inoculum was applied evenly over the foliage. Much of the suspension dripped off of the waxy surface of the foliage; that which remained tended to form droplets which were held at the junction of the needles and small shoots or were suspended from the lower sides of shoots. The foliage of the sixth plant, the control, received the same treatment using deionized water. The control plant and four of the inoculated plants were placed in inflated polyethylene bags immediately after inoculation to prevent the foliage from drying. The fifth inoculated seedling was not bagged, and the foliage dried within an hour after inoculation. The plants were maintained at room temperature (20–24 C) in the dark for the first 24 hr after inoculation.

After 24 hr, three of the inoculated plants and the uninoculated control plant were removed from their polyethylene bags and placed in an illuminated mist chamber where they were misted with deionized water for 20 sec every 4 min. The remaining bagged inoculated seedling was removed from its polyethylene bag and placed on a greenhouse bench (18–32 C) with the nonbagged inoculated plant. Every 24 hr thereafter, one of the inoculated plants was moved from the mist chamber to the greenhouse bench. The uninoculated control plant was removed with the last inoculated plant, 3 days after being placed in the mist chamber. The experiment was conducted twice.

**Pathogenicity of isolates from other hosts.** To determine whether the ability to cause foliar blight was unique to the isolate from redwood, three additional isolates of *P. citrophthora* and one isolate of *Phytophthora parasitica* Dastur were inoculated onto redwood foliage. The three isolates of *P. citrophthora*, each of which causes root rot on its respective host, were from *Penstemon* sp., *Ceanothus* sp., and *Citrus* sp. The isolate of *P. parasitica* causes root rot of Italian stone pine (*Pinus pinea* L.) (8).

The foliage of 6- to 8-mo-old plants was inoculated with zoospores as described in the preceding section. The concentrations of the suspensions ranged from $0.5 \times 10^5$ to $1 \times 10^5$ zoospores per milliliter. The plants were placed in inflated polyethylene bags and maintained at room temperature (20–24 C) in the dark for the first 24 hr after inoculation. The plants were then removed from their bags and placed on a greenhouse bench (18–32 C). An uninoculated control was included with each round of inoculations. Each seedling was inoculated with a single isolate; each isolate was tested twice.

**Inoculation of roots.** Ten 1-mo-old seedlings grown in UC-4 mix in 3-L plastic pots (one plant per pot) were inoculated by adding *P. citrophthora*-colonized millet seeds to the potting mix. The inoculum was prepared by autoclaving 100 g of dry millet seed in a 1-L flask for 60 min. One hundred milliliters of deionized water was added, and the mixture was autoclaved for another 60 min. After cooling, the sterilized millet was inoculated with an isolate of *P. citrophthora* from redwood and incubated in the dark at 20 C for 2 wk. A second flask containing uninoculated millet seed was prepared in the same manner for the inoculation of the control plants. During incubation, the flasks were shaken daily to facilitate the spread of the fungus and to prevent clumping of the seed and the formation of a solid mycelial mass.

Each seedling was inoculated with nine colonized millet seeds. The potting mix was infested by placing three seeds in each of three, 2.5-cm-deep holes positioned halfway between the seedling and the wall of the pot. The holes were then filled with potting mix. Ten control plants received the same treatment using noncolonized, autoclaved millet seed. After the potting medium was infested, the seedlings were placed on a greenhouse bench equipped with an automated watering system; a single heavy flow emitter was placed into each pot, and the plants were watered for 15 min three times a week.

Three months after inoculation, the plants were removed from the potting soil and examined visually for root rot. Samples of root tissue were plated onto PARPH, a medium selective for *Phytophthora* (6). The foliage and roots of the plants were dried separately in a forced-air oven at 80 C for 3 days and then weighed.

## RESULTS AND DISCUSSION

**Microscopic observation of zoospore encystment and infection of plant tissue.** When viewed under a dissecting microscope, zoospores were observed clustered on the surface of the redwood shoot pieces that had been floated in the zoospore suspension for 15 min. Examina-

tion of fixed tissue under the scanning electron microscope showed that encysted zoospores were clustered around stomata (Fig. 1A and B). Zoospores germinated within 1 hr after inoculation. The germ tubes of zoospores encysted near stomata grew directly into the stomatal openings (Fig. 1C). Many of the germ tubes formed appressoriumlike swellings before entering the stomata (Fig. 2A). Three to six hours after inoculation hyphae were growing out of the stomata, exiting from germinated cysts (Fig. 1D) or through stomata that had not been involved in the initial infection (Fig. 1E). The absence of exiting hyphae 1 hr after inoculation suggests that the exiting hyphae seen at 3 and 6 hr were not germ tubes, but rather grew from infected leaf parenchyma cells.

Zoospores that encysted on the leaf surface at sites other than near stomata were not observed to penetrate the leaf surface. Typically, encysted zoospores germinated and formed appressoria, which in turn produced unsuccessful infection hyphae on the cuticular surface of the leaf (Fig. 2B). These initial attempts at penetration were often followed by other unsuccessful attempts involving successively smaller appressoria. Our findings are in contrast to other foliar diseases caused by *Phytophthora* spp., such as late blight of potato or *P. megasperma* on soybean. The late blight fungus, *P. infestans* (Mont.) de Bary, enters the host via direct penetration of the periclinal wall of epidermal cells, as well as via stomata (3). *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin has been shown to penetrate the epidermis of soybean hypocotyls between anticlinal cell walls (10). The failure of zoospores of *P. citrophthora* to penetrate the surface of redwood foliage directly might be due to a thicker host cuticle or to the fungus's specialization as a root pathogen.

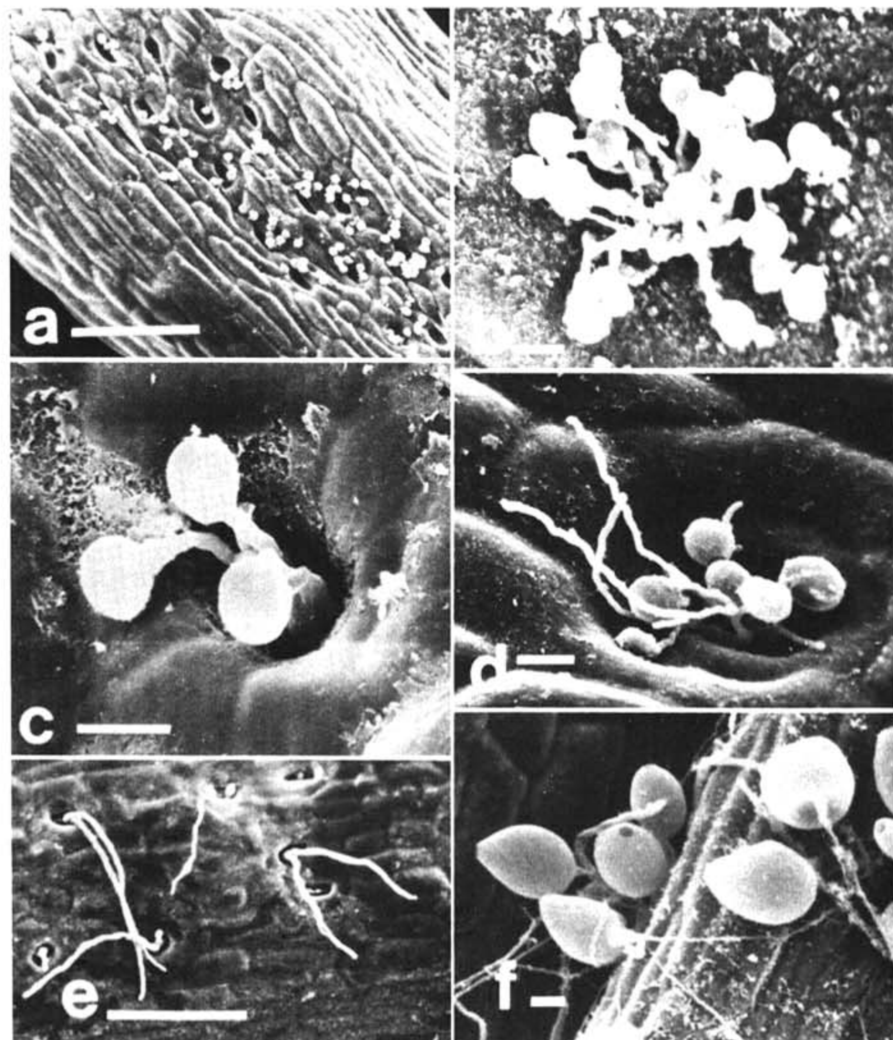

**Fig. 1.** Stages in the infection of giant redwood foliage by zoospores of *Phytophthora citrophthora*. **(A)** Encysted zoospores aggregated around the stomata of a leaf immediately after inoculation. Scale bar = 100 $\mu$m. **(B and C)** Germinated spores with germ tubes growing directly into stomata (1–3 hr after inoculation). Scale bars = 10 $\mu$m. **(D)** Hyphae emerging from a stoma that had been infected directly by zoospores (3–6 hr after inoculation). Scale bar = 10 $\mu$m. **(E)** Hyphae emerging from stomata that had not been infected directly by zoospores. Scale bar = 100 $\mu$m. **(F)** Sporangia of *P. citrophthora* on the surface of foliage. Space bar = 10 $\mu$m.

**Encysted zoospores aggregated around stomata.** Our studies were not critical enough, however, to determine if the swimming zoospores were attracted to the stomata, or if they were induced to encyst once they arrived at the stomata via random movement. Both possibilities are feasible given the high zoospore concentration of the inoculum. It is possible that both phenomena play a role in the observed aggregation. Zoospores of *Phytophthora* are known to be attracted to root surfaces and to specific compounds such as ethanol and amino acids (2). Zoospore encystment can be induced by surfaces or collisions or in response to factors such as extremes in pH or temperature (2). Additionally, autotaxis of encysted zoospores has been demonstrated (7); once a spore has encysted near a stoma, it may attract and induce the encystment of other swimming zoospores.

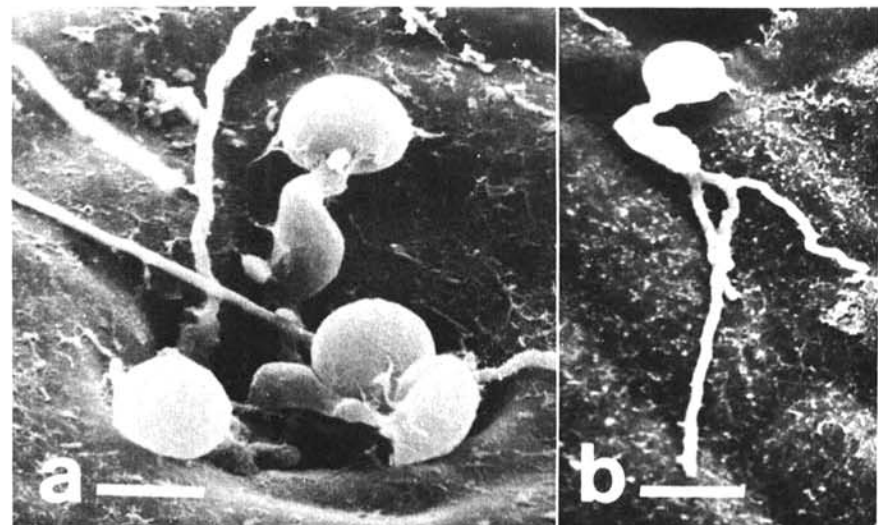

**Fig. 2.** Germinated zoospores with appressorialike structures. **(A)** An appressorium present at the invasion of a stoma. Scale bar = 10 $\mu$m. **(B)** An unsuccessful attempt at direct penetration of the leaf surface. Scale bar = 10 $\mu$m.

**The role of free moisture in disease**

**development.** No symptoms developed when foliage was inoculated with zoospores but not placed in a polyethylene bag to prevent desiccation. Placement of inoculated plants in polyethylene bags maintained free moisture on the foliage and resulted in severe disease. Water-soaked, gray-green lesions were observed on the wet foliage of these plants when they were removed from the bags 24 hr after inoculation. This infected tissue quickly died and became blackened and shiny. The foliage above the girdling lesions died more slowly, first wilting and then becoming dry and brown within 2 wk. This progression of symptoms matched those observed at the nursery.

Sporangia formed on the surface of the foliage of inoculated, bagged treatment plants within 16 hr after inoculation. These sporangia were mature and liberated zoospores within 20 min after being placed in chilled deionized water. Abundant, empty sporangia were also observed under a microscope on foliage from plants placed in the mist chamber 24 hr after inoculation. The zoospores released from these sporangia apparently caused secondary infections, since the longer the plants remained in the mist chamber, the greater the amount of foliage that was killed by direct infection rather than by dieback due to the girdling of stems.

**Table 1.** Effect of root inoculation with *Phytophthora citrophthora* on the growth of *Sequoiadendron giganteum* seedlings 3 mo after inoculation

| Treatment | Mean dry weights (g)[a] Roots | Shoots |
|---|---|---|
| Control | 13.27 (3.04) | 51.09 (10.08)[b] |
| Inoculated[c] | 10.59 (3.95) | 33.57 (15.24) |

[a]Values represent the means of 10 replicate seedlings per treatment. Numbers in parentheses are standard deviations of the means.
[b]Dry shoot weights from the two treatments were significantly different as determined by Student's $t$ test ($t = 3.03$, $P = 0.007$). Dry root weights for the two treatments were not significantly different.
[c]Seedlings were inoculated at 1 mo of age.

**Pathogenicity of isolates from other hosts.** All isolates tested, including the isolates of *P. citrophthora* from *Penstemon* sp., *Ceanothus* sp., and *Citrus* sp., and the isolate of *Phytophthora parasitica* from Italian stone pine, caused foliar blight. The ability of *P. citrophthora* to cause foliar blight on giant redwood, therefore, does not appear to be specific to the isolates originally obtained from redwood. Such nonspecific ability to cause foliar blight has been reported for *P. capsici* and *P. palmivora* on macadamia (1).

**Inoculation of roots.** *P. citrophthora* infected and killed roots of *S. giganteum* seedlings. Infected tissue was darkly discolored, and the margins of lesions were easily delineated from the white, healthy root tissue. The fungus was readily reisolated from symptomatic roots.

Root infection resulted in a significant reduction in foliar dry weight and a marked change in the morphology and distribution of the roots within the pots. Three months after inoculation the mean foliar dry weight of inoculated plants was 65.7% that of the controls (Table 1). While the difference in the mean dry root weights of the two treatments was not statistically significant, differences in the morphology and distribution of the roots were obvious. Compared to the controls, the roots of inoculated plants were thinner and more highly branched and were largely limited to the upper one-third of the pot in which they grew (Fig. 3). Such compensation following infection by *Phytophthora* species is common in the production of container-grown nursery stock and may be caused by the drier conditions present in the upper portions of the pots.

Because *P. citrophthora* causes root rot as well as the more obvious foliar blight may explain the observed failure of nursery trees to fully recover even after overhead irrigation was discontinued. This observation has practical ramifications in terms of disease control and the ethics of selling infected plants as living ornamentals. While diseased foliage can be removed by pruning, the eradication of *P. citrophthora* from infected roots is much more difficult, if not impossible.

**Fig. 3.** The effect of *Phytophthora citrophthora* on growth and distribution of the roots of 4-mo-old giant redwood seedlings that had been inoculated at 1 mo of age. The roots of the inoculated plant (left) were finer, more highly branched, and restricted to the upper third of the pot, as compared to the uninoculated control (right).

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