Fraser Fir Root Rot Induced by *Phytophthora citricola*

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ABSTRACT

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Phytophthora citricola was isolated from a Fraser fir seedling in a Christmas tree plantation in Avery County, NC. Pathogenicity was demonstrated on 1- and 4-yr-old fir seedlings in greenhouse tests using *P. citricola* zoospores and infested oat grain inoculum. Fir seedlings developed root necrosis, leaf chlorosis and necrosis, and wilting of new growth 10–14 days after inoculation. A zoospore concentration of 10^4 zoospores per plant caused 40% mortality of 1-yr-old fir seedlings. Propagules of *P. citricola* recovered from infested soil included sporangia, encysted zoospores, and oospores.

Root rot of Fraser fir (*Abies fraseri* (Pursh) Poir.) caused by *Phytophthora cinnamomi* Rands and *P. drechsleri* Tucker is a limiting factor in the production of Fraser fir as a Christmas tree species in western North Carolina (1,5,10). Severe losses occur in nurseries, where trees are grown for 5 yr, and in plantations, where trees are grown for an additional 6–9 yr. In routine isolations from Fraser fir seedlings exhibiting typical root rot symptoms, a different *Phytophthora* sp. was isolated. The fungus was identified as *P. citricola* Sawada based on characteristic morphological features (14–16).

P. citricola has been reported as a root rot and dieback pathogen of hybrid rhododendron (7,8) and *Pieris japonica* (3), a root rot pathogen of black walnut (6), and a minor root pathogen of avocado (16). In this study we examined the pathogenicity of *P. citricola* on Fraser fir.

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MATERIALS AND METHODS

The isolate of *P. citricola* used was obtained from the stem of a Fraser fir seedling in Avery County, NC, that was exhibiting foliar chlorosis and necrosis. Cultures were maintained on 5% clarified V-8 juice agar (7) at 20 and 25 C and transferred at least every 2 mo.

Fir seedlings were obtained from two sources. One-year-old seedlings were grown from seed in the greenhouse at 18–25 C. Seeds were placed in Rootrainer trays (Spencer-Lemaire Ind., Edmonton, Alberta, Canada) containing Metro mix 220 (W. R. Grace and Co., Cambridge, MA). After germination, seedlings were fertilized every 2 wk from February to August with Peters 20-20-20 soluble fertilizer (R. B. Peters Co., Allentown, PA). Four-year-old seedlings were obtained from the North Carolina Forest Service Fraser fir nursery at Crossnore, NC.

We used oat grains infested with *P. citricola* as inoculum in initial pathogenicity tests. Agar disks (9 mm diam) of 3-day-old cultures of *P. citricola* growing on V-8 juice agar were placed on sterile oat grains in 500-ml flasks (75 cc of oat grains plus 50 ml of distilled H_2O/flask). After 3-wk incubation at 25 C, the oat grains were mixed into sand, soil, and peat (1:1:1 by volume) to give about 40 oat grains per 10-cm-diameter clay pot of soil mix.

We wounded some plants and pruned the roots of others before transplanting them into infested soil to determine if wounding was required for disease development. Wounding was accomplished by removing a strip of bark about 15 mm long and 3 mm wide in the hypocotyl region of the stem. Roots were pruned by removal of about one-third of the root system. Other plants were not wounded

or root pruned but were transplanted into infested soil. Control plants were wounded or root pruned and transplanted into noninfested soil.

A single 4-yr-old Fraser fir seedling was transplanted into each pot. The pot was placed in a saucer to maintain a 2–3 cm saturated layer of soil in the bottom of the pot. Seedlings were maintained in the greenhouse at 25–35 C and observed daily for symptoms.

We also conducted pathogenicity tests with *P. citricola* zoospores as inoculum. Cultures were started by placing three 4-mm-diameter agar disks from the margin of a 3-day-old V-8 juice agar culture of *P. citricola* in a 9-cm petri dish containing 16–20 ml of 10% clarified lima bean extract broth. After 3 days of growth at 25 C, the broth was removed and the mycelial mats rinsed twice with sterile distilled water. Cultures were incubated for 30–60 min in sterile distilled water before being drained and the water replaced with 15 ml of sterile, Chen-Zentmyer salt solution (2) as modified by Rao et al (12). The ferrous ion was omitted from the salt solution because it was inhibitory to sporangial formation.

Cultures were incubated in salt solution for 2 days at 25 C in continuous light, then rinsed three times and covered with 15 ml of sterile distilled water. Zoospores were released when cultures were chilled for 30 min at 6 C then returned to room temperature for 30–60 min. Spore encystment was induced by placing three drops of a 2% cotton blue solution in 5 ml of spore suspension. Spore counts in 10 fields of a hemacytometer were averaged to determine spore concentration.

One-year-old Fraser fir seedlings were transplanted into vermiculite in 6-cm-diameter clay pots. Pots were placed in saucers and maintained in the greenhouse at 25–35 C. After 2 wk, pots were submerged in pans 15 cm deep containing enough water to maintain the water level 0.5 cm above the vermiculite (11). Zoospores at densities of 1×10^4, 2.5×10^4, 5×10^4, and 1×10^5 per pot were added with a wide-mouth pipette to 10 pots per zoospore density. Pots were maintained in the submerged condition for 10 min and then returned to water-filled saucers that allowed the water level to drain to about one-third the height of the pot (2–3 cm). Plants were observed daily for symptoms. After 45 days, we assayed the entire root system and stem of each seedling on a medium selective to *Phytophthora* to determine the percentage of plants infected (13).

RESULTS AND DISCUSSION

Fraser fir seedlings inoculated with *P. citricola* developed foliar symptoms including chlorosis, wilting of new growth, and necrosis within 14 days after being transplanted into infested soil. Root symptoms were severe, often with more than 90% of the roots necrotic. Symptoms were the same as those described for Fraser fir seedlings inoculated with *P. cinnamomi* and *P. drechsleri* (1,5,10).

Hypocotyl wounding had no effect on mortality of seedlings. Mortality was 100% after 19 days in both wounded and unwounded seedlings. Root pruning before inoculation resulted in 100% infection, but mortality was only 50% after 45 days. Root systems of the root-pruned seedlings were more than 50% necrotic when assayed.

One-year-old fir seedlings died as early as 10 days after inoculation with zoospores of *P. citricola*. Infection was 70, 80, 90, and 80% at zoospore densities of 1×10^4, 2.5×10^4, 5×10^4, and 1×10^5 zoospores per plant, respectively. Mortality was 40, 60, 70, and 60% at these respective densities. Similar numbers of zoospores caused 50% mortality on tobacco (4), papaya (9), and milkweed vine and watercress (11) with four different *Phytophthora* spp.

After the 45-day incubation period, soil from the pathogenicity tests using oat grain inoculum was plated on the selective agar medium to determine what propagules of *P. citricola* were present in the soil. After 48 hr of incubation at 24 C, the soil was washed from the agar surface and the plates observed microscopically. Encysted zoospores, sporangia, oospores, and infested organic matter gave rise to colonies of *P. citricola* on the agar medium. Zoospores and sporangia were the most prevalent structures observed, but oospores were also present (many in the organic matter fraction). Zoospores and sporangia of *Phytophthora* spp. may survive in soil for several weeks or months but are not considered long-term survival structures. As a homothallic fungus, *P. citricola* produces and thus may survive in soil as oospores. Hence, even long-term rotations may not be adequate for eliminating *P. citricola* inoculum from the soil.

Our results demonstrated the pathogenicity of *P. citricola* to Fraser fir seedlings under greenhouse conditions. Nonetheless, losses to *P. citricola* in Fraser fir nurseries and plantations are considered minor at present based on isolations made by the Plant Disease and Insect Clinic at North Carolina State University. A less prevalent geographic distribution in Fraser fir plantations may account for the minor importance of *P. citricola* on Fraser fir when compared with *P. cinnamomi* and *P. drechsleri*. *P. citricola*, like *P. drechsleri* (1), is considered a warm-soil pathogen, which may also limit its importance in Fraser fir soils where temperatures are only infrequently above 25 C. Movement of infected nursery stock and an increasing tendency to grow Fraser fir outside its natural range may increase the prevalence of *P. citricola* root rot as well as that caused by other *Phytophthora* spp. in Christmas tree plantings.

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