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First Report of *Phytophthora ramorum* Infecting Grand fir in California

Kathleen L. Riley and **Gary A. Chastagner,** Department of Plant Pathology, Washington State University Research & Extension Center, 2606 West Pioneer, Puyallup, WA 98371; and **Cheryl Blomquist,** Senior Plant Pathologist, Plant Pest Diagnostics Branch, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832

Corresponding author: Kathleen L. Riley. klriley@wsu.edu

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Phytophthora ramorum, the oomycete that causes sudden oak death, was first detected on grand fir (*Abies grandis*) in a Christmas tree plantation near Los Gatos, CA, in January 2003. Symptoms included wilting and dieback of 2002 shoot growth shortly after bud break, followed by lesion development that extended into year-old wood. In 2005, infection of new growth was observed at the time of shoot elongation in mid-May (Fig. 1). In both years, infected trees were located in close proximity to *P. ramorum*-infected California bay laurel (*Umbellularia californica*) (Fig. 2).

Fig. 1. Shoot wilting, needle loss, and lesion development on grand fir in a Christmas tree plantation near Los Gatos, CA.

Fig. 2. Symptoms of *Phytophthora ramorum* infection on grand fir Christmas trees growing under infected California bay laurel.

Isolations from branch samplings onto PARP and CARP (corn meal agar amended with ampicillin (trihydrate, 200 ppm), rifampicin (10 ppm), and pimaricin (5 ppm) media in 2003 and 2005 yielded *P. ramorum*, which was identified based on morphological characteristics (2) and PCR (1). To confirm the susceptibility of grand fir to this pathogen, inoculation experiments were conducted in both years at APHIS-approved facilities.

In 2003, an isolate from grand fir was grown on V8 agar for 10 days at 18°C. Stems of five, 1-year-old grand fir seedlings were wounded and inoculated with colonized agar plugs from this isolate shortly after bud break, approximately 5 cm from the plant apex, and wrapped with Parafilm to prevent dehydration. Five control seedlings received plugs of V8 agar only. Plants were grown at 18°C in a growth chamber and plugs were removed after 7 days. Over time, tips of the

infected shoots wilted, became necrotic and were eventually girdled. After 3.5 months, lesions measured from 5.2 to 10.4 cm. Lesions measured from the point of wounding on the controls ranged from 2 to 7 mm. Small pieces from the edge of the lesions from each seedling were plated onto PARP and monitored for growth for 2 weeks at 18°C. *P. ramorum* grew from lesions from each of the inoculated seedlings. No *P. ramorum* was isolated from the controls.

In 2005, another grand fir isolate of *P. ramorum* collected from the same location was used to inoculate grand fir seedlings that had been forced in a greenhouse to bud break of 1 to 3 cm. The isolate was grown on V8 agar and incubated in the dark at 19°C for 35 days to induce sporangia/zoospore production. Cultures were flooded with sterile, distilled water and the zoospore suspension (6.53×10^5 spores/ml sterile water) was misted onto unwounded shoots of six seedlings using an artist's airbrush. Six control seedlings were sprayed with water alone. High relative humidity was maintained by placing seedlings in plastic tubs over 2.5 cm of water and covered with a plastic bag supported by a wire frame. Tubs were placed in a growth chamber at 15 to 20°C with 24 h light. After 5 days, covers were removed and the seedlings were maintained at the same temperature and light conditions.

After 13 days, new growth had wilted, shoots had died back and stem lesions had developed (Fig. 3). Symptomatic tissue isolated onto CARP medium yielded *P. ramorum* from all six inoculated seedlings. No symptoms developed on any of the control plants. These results confirm grand fir as a host of *Phytophthora ramorum*. The potential for infection within its native range is unknown.

Fig. 3. Wilting and lesion development on a grand fir seedling inoculated with a zoospore suspension of *P. ramorum*.

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