Diseases Caused by Oomycetes

First Report of Leaf Blight Caused by *Phytophthora ramorum* on Cherry Laurel (*Prunus laurocerasus*) in Washington State, U.S.A.

M. Elliott,[†] L. Rollins, T. Bourret, and G. Chastagner

Department of Plant Pathology, Washington State University Research and Extension Center, Puyallup, WA 98371

Funding: This work was supported by the USDA National Institute of Food and Agriculture, McIntire-Stennis project 1019284 and USDA APHIS Cooperative Agreement AP17PPQS&T00C070. Plant Dis. 105: 712, 2021; published online as <https://doi.org/10.1094/PDIS-07-20-1489-PDN>. Accepted for publication 5 October 2020.

In April 2014, *Phytophthora ramorum* (Werres, De Cock & Man in't Veld) was recovered from symptomatic foliage of cherry laurel (*Prunus laurocerasus*) at an ornamental plant nursery in Washington State. Cherry laurel, also known as English laurel, is widely propagated in Washington State because it is commonly used in landscaping. It is invasive in forests near the urban/wildland interface in the western United States and in Europe. Given its popularity as an ornamental species, the potential of this host to spread *P. ramorum* is of regulatory concern due to possible long-distance spread to other states via nursery stock. Foliar symptoms consisted of dark brown lesions near wounds or around leaf margins where water collected. Shot-hole symptoms characterized by abscission zones and dropping of infected tissues were also observed. Lesions expanded beyond the margin of the shot-hole in some cases. *Phytophthora* was isolated from symptomatic foliage by surface sterilizing leaf pieces in 0.6% sodium hypochlorite and two rinses in sterile water. They were plated on PARP medium (Ferguson and Jeffers 1999). After 2 to 3 days, a slow-growing dense colony with coraloid hyphae was isolated onto V8 agar. *P. ramorum* was identified by observing morphological features. Colony and spore morphology matched that of *P. ramorum* (Werres et al. 2001). The isolate was confirmed as *P. ramorum* by PCR and sequencing of ITS and COX1 regions using primers ITS1/ITS4 (White et al. 1990) and COX1F1/COX1R1 (Van Poucke et al. 2012). Sequences were submitted to GenBank (accession nos.: ITS, MT031969; COX1, MT031968). BLAST results showed at least 99% similarity with sequences of *P. ramorum* (ITS,

KJ755124 [100%]; COX1, EU124926 [99%]). Multilocus genotyping with microsatellite markers placed the isolate in the EU1 clonal lineage. Pathogenicity of *P. ramorum* on cherry laurel was confirmed by completing Koch's postulates using the isolate taken from this host. Two trials were done in a biocontainment chamber (USDA-APHIS permit no. 65857), because *P. ramorum* is a quarantine pathogen and greenhouse trials could not be conducted, using detached stems from mature, visibly healthy cherry laurel plants growing in a landscape. *P. ramorum* inoculum was grown on V8 agar plates at 20°C for 2 weeks until sporangia were abundant. A zoospore suspension was produced by flooding plates with 7 ml of sterile water and then incubating for 2 h at 5°C followed by 1 h at 24°C. Zoospores were observed with light microscopy, quantified with a hemocytometer, and diluted to 1×10^4 zoospores/ml. A 10- μ l droplet was placed at three wounded and three unwounded sites on four leaves per branch. In addition, a set of samples was inoculated by dipping foliage into the zoospore suspension for 30 s. A set of controls was mock inoculated using sterile water. Four branches per inoculation treatment were used, and the trial was repeated once. Inoculated plant materials were incubated in moist chambers for 3 to 5 days at 20°C. Free moisture was present on foliage upon removal. Symptom development was assessed after incubation in the biocontainment chamber at 20°C for 7 days. *P. ramorum* was reisolated from symptomatic tissue, and the recovered culture was verified morphologically and by PCR and sequencing. It was isolated more often from foliage dipped in zoospore suspension than droplet-inoculated leaves, and more from wounded than unwounded sites. None of the water-inoculated controls were positive for *P. ramorum*. The presence of *P. ramorum* was also confirmed with DNA extraction from surface-sterilized symptomatic foliage followed by PCR and sequencing of the COX1 gene (EU124926, 100%). To our knowledge, this is the first report of *P. ramorum* naturally infecting cherry laurel in the United States.

References:

- Ferguson, A. J., and Jeffers, S. N. 1999. Plant Dis. 83:1129.
- Van Poucke, K., et al. 2012. Fungal Biol. 116:1178.
- Werres, S., et al. 2001. Mycol. Res. 105:1155.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: ornamentals, woody ornamentals, oomycetes, invasive species

[†]Indicates the corresponding author.
E-mail: M. Elliott; melliott2@wsu.edu