Diseases Caused by Oomycetes

First Report of Leaf Blight Caused by *Phytophthora ramorum* on Periwinkle (*Vinca minor*) in Washington State, U.S.A.

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Phytophthora ramorum (Werres, De Cock & Man in't Veld) was recovered from symptomatic foliage of periwinkle at a botanical garden in Washington (WA) in March 2015. Symptoms were tan-colored lesions with a dark brown margin visible on both surfaces of the leaf and were found on wounds or around leaf margins. Periwinkle is native to Europe and is commonly used for ground cover in ornamental landscapes. It is known to be invasive in U.S. forests near the urban/wildland interface. Potential spread of *P. ramorum* into WA forests is of regulatory concern, as well as long-distance spread to other states via nursery stock (Title 7, Code of Federal Regulations, Part 301.92-2). *P. ramorum* was isolated from symptomatic foliage by excising leaf pieces 4 to 6 mm in diameter and surface sterilizing in 0.6% sodium hypochlorite followed by two rinses in sterile water. Leaf pieces were plated on PARP medium (Ferguson and Jeffers 1999), and after 2 to 3 days at 20°C, slow-growing dense colonies with coraloid hyphae were isolated onto V8 agar. Colony morphology and chlamydospore production were consistent with descriptions of *P. ramorum* (Werres et al. 2001), except that the isolate was slower growing and had irregular, non-wild-type morphology (Elliott et al. 2018) compared with other isolates of *P. ramorum*. ITS and COX1 regions of mycelial DNA were amplified and sequenced to confirm the identity of *P. ramorum* using primers ITS1/ITS4 (White et al. 1990) and COX1F1/COX1R1 (Van Poucke et al. 2012). Sequences were submitted to GenBank (accession nos.: ITS, MT031975; COX1, MT031974). BLAST results showed at least 98% similarity with sequences of *P. ramorum* (ITS, MN540640 [98%]; COX1, EU124920 [100%]) and belonged to the

NA1 clonal lineage. Pathogenicity of *P. ramorum* to periwinkle was confirmed by completing Koch's postulates. 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 incubating for 2 h at 5°C followed by an additional hour at 24°C. Zoospores were observed under the microscope, quantified with a hemocytometer, and then diluted to 2×10^5 zoospores/ml. A 10-μl droplet of inoculum was placed at one wounded and one unwounded site on six leaves on each of four plants. In addition, a set of four plants was inoculated by dipping foliage on one branch per plant into the zoospore suspension for 30 s. A set of four control plants were mock inoculated in the same manner using sterile water. The trial was repeated once. Inoculated plant materials were incubated in a moist chamber for 3 to 5 days, and free moisture was present on foliage upon removal. Plants were held in a biocontainment chamber (USDA-APHIS permit no. 65857) at 20°C and symptom development assessed after 7 days. Symptoms developed on foliage inoculated using both methods in both trials. *P. ramorum* was isolated once from droplet-inoculated foliage at a wounded site on one plant. Reisolation onto PARP and then V8 agar was conducted from surface-sterilized symptomatic tissue and the presence of *P. ramorum* confirmed by observation of colony morphology and chlamydospore production. The presence of *P. ramorum* was also confirmed with DNA extraction from symptomatic foliage from plants from each of the two trials followed by PCR and sequencing of the COX1 gene (EU124920, 100%). None of the water-inoculated controls were positive for *P. ramorum*. Low isolation success could be attributed to reduced pathogenicity due to being a non-wild-type isolate.

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The author(s) declare no conflict of interest.

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