Diseases Caused by Oomycetes

First Report of *Phytophthora litorale* Causing Crown and Root Rot on *Rhododendron pulchrum* in China

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During a survey of pathogenic oomycetes in Nanjing, China, from 2019 to 2020, 10 adjacent *Rhododendron pulchrum* plants at a Jiangjun Mountain scenic spot showed symptoms of blight and crown and root discoloration. Symptomatic root tissues were collected, rinsed with water, cut into 10-mm pieces, surface sterilized with 70% ethanol for 1 min, and plated onto 10% clarified V8 PARP agar (cV8A-PARP) containing pimarcin (20 mg/liter), ampicillin (125 mg/liter), rifampicin (10 mg/liter), and pentachloronitrobenzene (20 mg/liter). Four *Pythium*-like isolates were recovered after 3 days of incubation at 26°C and purified by hyphal tipping. Ten plugs (2 mm × 2 mm) of each isolate were grown in 10 ml of 10% cV8 juice for 3 days to produce mycelial mats, and then cV8 was replaced with sterile water. To stimulate sporangial production, five drops of soil extract solution were added to each plate. Sporangia were terminal, ovoid to globose, 24 to 45.6 µm (mean 34.7 µm) ($n = 20$) in length × 23.6 to 36.0 µm (mean 29.8 µm) ($n = 20$) in width. The isolates were subcultured on cV8A-PARP, potato dextrose agar, and corn meal agar, and all isolates had identical morphological features. Complete ITS, partial LSU, and *cox2* gene regions were amplified using primer pairs ITS1/ITS4 (White et al. 1990), NL1/NL4 (O'Donnell 1993), and FM58/FM66 (Martin and Tooley 2003), respectively. The ITS, LSU, and *cox2* sequences of isolate PC-dj1 (GenBank nos.

MW205746, MW208002, and MW208003) were 100.00% (936/936 nt), 100.00% (772/772 nt), and 99.64% (554/556 nt) identical to those of JX985743, MT042003, and GU133521, respectively. Interspecific differences were observed in a maximum-likelihood tree of *Phytophthora* species using the concatenated dataset (ITS, LSU, and *cox2*). Based on morphology and sequence data, PC-dj1 was identified as *Phytophthora litorale* (Nechwatal et al. 2006). The pathogenicity of PC-dj1 was tested using two inoculation methods on 10 one-year-old *R. pulchrum* plants. In the first method, plants were removed from the pot, and roots were rinsed with tap water to remove soil. Five plants were each placed in 250 ml of sterile water with 10 blocks (10 mm × 10 mm) of mycelial mats from a 3-day-old culture of *P. litorale*, and five plants were placed in sterile water (control). Symptoms of crown rot, root rot, and blight were observed on inoculated plants, whereas control plants were asymptomatic after 3 days. For the second method, 10 plants were dug up. Ten 3-day-old cV8A plugs (5 mm × 5 mm) of a PC-dj1 culture were evenly inserted into the root ball of a plant before it was replanted in the original pot. Plants were maintained in a growth chamber at 26°C with a 12-h/12-h light/dark cycle and irrigated as needed. After 21 days, inoculated plants had symptoms resembling those in the field, whereas control plants remained asymptomatic. Each method was repeated thrice with identical outcomes. Isolates with morphology and sequences identical to those of PC-dj1 were recovered from rotted crown and root tissues of all inoculated plants. Previously, *P. litorale* was reported causing disease on apple and *Platanus orientalis* in Turkey (Derviş et al. 2020; Mert et al. 2020) and seedling damping-off of yellow squash in southern Georgia, U.S.A. (Parkunan and Ji 2013). This is the first report of *P. litorale* causing crown and root rot on *R. pulchrum*, an important ornamental plant species in China.

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