Diseases Caused by Oomycetes

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

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During a 2019 to 2020 survey of plant-pathogenic oomycetes in Nanjing, China, five adjacent *Rhododendron pulchrum* plants in Xuanwuhu Park exhibited symptoms including crown and root rot and wilting. Diseased roots were rinsed in water, cut into 10-mm pieces, immersed in 70% ethanol for 60 s, and plated onto clarified V8 juice (cV8) agar amended with pimarin (20 mg/liter), ampicillin (125 mg/liter), rifampicin (10 mg/liter), and pentachloronitrobenzene (20 mg/liter). After 3 days of incubation at 26°C, five *Pythium*-like isolates were obtained using hyphal tipping. Ten agar plugs (2 × 2 mm²) of each isolate were transferred into 10 ml of 10% cV8 in a 100-mm plate at 26°C to produce mycelial mats. After 3 days, cV8 was replaced with sterile water. To stimulate sporangial production, three to five drops of soil extract solution were added to each plate. Five isolates had identical morphological features. Sporangia were terminal, ovoid to globose, measuring 34.2 ± 6.2 µm (24.0 to 42.5 µm range) in length and 30.7 ± 6.6 µm (20.9 to 41.1 µm range) in width. Oogonia were not observed. The rDNA internal transcribed spacer (ITS)

region and the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and 2 (*cox2*) genes of representative isolate PH-C were amplified using the primer pairs ITS6 and ITS4 (Cooke et al. 2000), OomCoxI-Levup and OomCoxI-Levlo (Robideau et al. 2011), and Cox2-F (Hudspeth et al. 2000) and Cox2-RC4 (Choi et al. 2015), respectively. A representative isolate, Phe-1, had identical sequences of the rDNA ITS region (675 nt) and the mitochondrial cytochrome c oxidase subunit 1 (657 nt) and 2 (561 nt) genes (GenBank MT824568, MT834959, and MT834958, respectively) to those of *Phytophthora helicoides* (MN541109, MK879709, and KT595689, respectively). Based on the morphological and molecular characters, the causal agent was identified as *P. helicoides*. One-year-old *R. pulchrum* plants (approximately 0.3 m in height) grown in 8 × 8-cm pots were used in pathogenicity trials. Ten plants were carefully dug up to expose root balls. Three-day-old cultures of isolate PH-C were used as the inoculum. Five plants were inoculated by inserting 10 agar plugs into the root ball of each plant. For five control plants, sterile cV8 agar plugs were used. All plants were repotted using the original potting mix and pots. Plants were maintained in a growth chamber set at 26°C with a 12-h/12-h light/dark cycle and irrigated as needed. After 21 to 25 days, the inoculated plants had symptoms identical to those in the field, whereas the controls remained asymptomatic. Identical outcomes were obtained from two repeated pathogenicity trials. *P. helicoides* was reisolated from all symptomatic plants in the trials. *P. helicoides* was found causing diseases of Asian lotus (Yin et al. 2016), mandarin orange (Chen et al. 2016), and kiwifruit (Wang et al. 2015) plants in China. Globally, this is the first report of *P. helicoides* causing crown and root rot of *R. pulchrum*. Additional surveys are being conducted to map the distribution of *P. helicoides* in Nanjing, China.

References:

- Chen, X. R., et al. 2016. Eur. J. Plant Pathol. 146:715.
- Choi, Y. J., et al. 2015. Mol. Ecol. Resour. 15:1275.
- Cooke, D. E. L., et al. 2000. Fungal Genet. Biol. 30:17.
- Hudspeth, D. S., et al. 2000. Mycobiology 92:674.
- Robideau, G. P., et al. 2011. Mol. Ecol. Resour. 11:1002.
- Wang, K. X., et al. 2015. Plant Dis. 99:725.
- Yin, X., et al. 2016. Plant Dis. 100:532.

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