Diseases Caused by Oomycetes

First Report of *Phytophthora vexans* Causing Root and Crown Rot on Flowering Cherry in Tennessee

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Flowering cherry (*Prunus serrulata* Lindl. 'Kwanzan') rooted cuttings grown in propagation beds containing 40% coarse sand and 60% ground pine bark in a commercial propagation nursery in Warren County, Tennessee, were exhibiting root and crown rot in December 2016. Dark brown to black soft lesions were observed in the roots as well as the crown region of flowering cherry rooted cuttings, and those rooted cuttings were non-marketable due to lesions. Disease incidence was approximately 60% of 10,000 plants. *Phytophthora* ImmunoStrip test (Agdia, Elkhart, IN) was performed, and the test result was positive. Diseased plant tissues were surface sterilized with 70% ethanol and washed twice with distilled water. Culturing the affected root and crown parts (1-cm pieces) on V8-PARPH, an oomycete-selective medium, consistently yielded whitish radiate mycelial growth pattern with spherical zoospores, filamentous to globose oogoni, elongated, and cylindrical antheridia with constrictions (de Cock et al. 2015) after 7 days of incubation at 25°C in a 12-h fluorescent light and dark cycle, which is the typical morphology of *Phytophthora vexans* (de Bary) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque. To confirm pathogen identity, total DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) directly from a 3-day-old culture of isolate FBG2017010 on V8 medium. The internal transcribed spacer (ITS) and 28S large subunit of ribosomal RNA and the cytochrome c oxidase

subunit I (CoxI) of mitochondrial DNA (mtDNA) genes/ region were amplified by PCR using the primer pairs ITS1/ITS4 (White et al. 1990), NL1/NL4 (Baten et al. 2014), and Levup and Fm85mod (Robideau et al. 2011), respectively. The PCR products were sequenced, and the sequences (GenBank accession nos. MT533275, MT533451, and MT547980) were compared with the voucher specimens. They were 99.23, 99.60, and 98.92% similar to those of *P. vexans* isolates in the NCBI database (HQ643400, KR092144, and HQ708996, respectively). To complete Koch's postulates, Kwanzan flowering cherry rooted cuttings grown on propagation substrate (10-cm pot containing 1 kg of sterilized 40% coarse sand and 60% ground pine bark) were inoculated with identified pathogen, and observations were taken on root rot disease symptoms. Five plants were inoculated with 100 ml of pathogen agar-slurry (one plate of a 7-day-old culture of isolate FBG2017010/1 liter of sterilized water), and five control plants were drenched with agar slurry. The plants were maintained in the greenhouse condition (day/night temperature of 26/24°C) and irrigated twice a day for 2 min by an overhead irrigation system. After 2 weeks, dark brown to black necrotic root lesions developed on all inoculated cuttings, and *P. vexans* was consistently reisolated from the inoculated plants. The morphology of the pathogen isolated on the V8-PARPH medium was identical to the original isolate. All control plants remained symptom-free, and *P. vexans* was not isolated from the root tissue. To our knowledge, this is the first report of *P. vexans* causing root and crown rot in Kwanzan flowering cherry in Tennessee, which can be a potential threat for the nursery crop production. The identification of *P. vexans*, the causal agent of *Phytophthora* root and crown rot, is important in determination and implementation of effective management strategies.

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