Diseases Caused by Oomycetes

First Report of *Pythium ultimum* Associated with Peony Root Rot in China

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Peony (*Paeonia suffruticosa* Andr.) is a well-known ornamental and aromatic plant with hundreds of cultivars in China. The root cortex of *P. suffruticosa* is used in traditional Chinese medicine, and the seed oil has been found to be rich in unsaturated fatty acids, amino acids, and stilbenoids. During August 2018, a severe outbreak of root rot was observed in a commercial field located in Nanyang, Henan Province, P.R. China, on *P. suffruticosa*. Ten percent of plants were affected and showed stunting and extensive chlorosis of the leaves. Root systems and collars of diseased plants appeared rotted, and in severe cases, the plants died. Ten arbitrary symptomatic plants were removed from the soil, and brown, water-soaked root lesions were observed. The roots of the plants were rinsed in tap water to remove soil debris, and symptomatic tissue fragments (3 mm²) were excised from the roots of infected plants, dipped in a solution containing 1% sodium hypochlorite for 3 mins, rinsed with sterile water, dried on a paper towel, and then plated on 2.5% vegetable (V8) juice medium. The plates were incubated at 25°C for 4 to 5 days in the dark. White fungal colonies grew from the tissue pieces. Single hyphal tip cultures were placed on corn meal agar containing rifampicin (50 mg/liter), phenamacril (5 mg/liter), ampicillin (50 mg/liter), and pentachloronitrobenzene (50 mg/liter) (Chen et al. 2017). Five isolates with similar morphological characteristics were obtained, and observations under a light microscope showed hyphae that were aseptate, ranged from 3.3 to 8.7 µm in diameter, and produced sporangia consisting of complexes of swollen hyphal branches. Oogonia were globose, terminal, smooth, and 18.7 to 30.2 (average 24.6) µm in diameter. Antheridia were monoclinal, extending from immediately below the oogonium, and measured 9.6 to 18.2 × 7.2 to 13.8 (average 13.9 × 10.5) µm. Oospores were single, globose, aplerotic, thick walled, and 16.8 to 22.2 (average 19.5) µm in diameter, with a 1.1- to 2.1-µm-thick wall. Zoospores were produced only in sterile water grass blade cultures and were reniform and biflagellate. The isolates were identified as *Pythium* spp. based on growth pattern and sporangial and

oogonial structures (Van der Plaats-Niterink 1981). For the molecular identification, polymerase chain reaction was performed using the primers ITS1/ITS4 (White et al. 1990) to amplify the internal transcribed spacer (ITS) region of rDNA of the five isolates, and these products were directly sequenced. ITS sequences of the isolates were identical, and a single sequence deposited in GenBank (accession no. MN421544) shared 99.89 to 99.77% (zero to two substitutions and one to two gaps out of 874 nt) similarity with *Pythium ultimum* isolates in GenBank (accession nos. AB355596.1, MK794638.1, AY598657.1, and others). The *COI* gene was amplified with the following primers: OomCoxI-Levlo/OomCoxI-Levup (Robideau et al. 2011). The *COI* nucleotide sequences of the isolates were also identical, and a single sequence has been assigned GenBank accession number MN431954, and shared 99.86 to 99.29% (one to five substitutions out of 707 nt) similarity with *P. ultimum* isolates in GenBank (accession nos. KJ639183.1, GU138663.1, KJ639191.1, and others). Phylogenetic analysis was done by neighbor-joining analysis based on ITS + *COI* sequences. The isolates were identified as *P. ultimum* based on molecular analysis and morphological characteristics. Pathogenicity of each isolate was tested on peony under greenhouse conditions (30°C day and 20°C night). Ten 12-week-old peony seedlings (cv. FengDanBai) grown in 20-cm pots (sterilized field soil) were inoculated with the isolates separately by applying 1 ml of *P. ultimum* spores (1 × 10⁴ CFU/ml) (Green and Jensen 2000) and mycelium pipetted around the base of the stem of each seedling. Ten noninoculated peony seedlings were used as the control. At 30 days after planting, the inoculated peony plants developed collar and root rot, with some leaf chlorosis, similar to symptoms observed in the field. Symptoms did not develop on the control plants. *P. ultimum* was consistently isolated from the roots. To our knowledge, this is the first report of *P. ultimum* causing disease of peony in China as well as worldwide. Henan is one of the important places of peony cultivation in China, and the occurrence of root rot caused by *P. ultimum* needs to be taken into account in developing disease management programs in cultivation.

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