Diseases Caused by Oomycetes

First Report of *Pythium sylvaticum* Causing Corn Root Rot in Northeastern China

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As one of the most planted crops worldwide, corn has continuously increased in importance in China over the last decade. But in recent years, poor stands of corn seedlings have occurred frequently in northeastern China, causing significant economic loss. Mature plants were stunted, the roots were necrotic, and some plants collapsed. We collected soil samples from five fields with a history of poor stands of corn seedlings in the Heilongjiang province of China in October 2017. After being planted in the collected soil for 12 days, corn seedlings were uprooted. The pathogen was then isolated as described by Tang et al. (2019). Briefly, the rotted roots were washed in 0.5% NaOCl for 2 min, rinsed in sterile water, and then cut into 1- to 2-mm segments and placed on cornmeal agar amended with pimaricin (5 µg/ml), ampicillin (250 µg/ml), rifampicin (10 µg/ml), pentachloronitrobenzene (50 µg/ml), and benomyl (10 µg/ml) (PARP+B), which is selective for oomycetes (Jeffers and Martin 1986). After 3 days of incubation in the dark at 25°C, colonies were transferred to 10% V8 juice agar or potato dextrose agar (PDA) and grown for 7 days at 25°C. Based on morphological characteristics, one putative isolate (COPS) was identified as *Pythium sylvaticum* (Campbell and Hendrix 1967). On PDA, the culture (COPS) produced creamy white and floccus mycelium. *P. sylvaticum* (COPS) produced hyphal swellings but no oogonia or zoospore. Hyphal

swellings were globose, terminal, or intercalary, ranging from 12.22 to 18.55 µm in diameter. Sequence analysis was performed with the cytochrome c oxidase subunit II (*COII*) gene amplified with primers FM35/FM52 (Martin 2000) and the rDNA ITS amplified with primers DC6/ITS4 (Cooke et al. 2000). For *COII* gene, BLAST analyses of the 773-bp segments showed 97.93% identity with a *P. sylvaticum* isolate (GenBank accession no. GU222164.1). For the ITS, BLAST analyses of the 880-bp segments showed 99.89% identity with a *P. sylvaticum* isolate (GenBank accession no. KY084736.1). Both sequences were submitted to GenBank with accession numbers MK648400 and MK606071 for *COII* and ITS, respectively. For pathogenicity tests, similar to that described by Ling et al. (2018), four 9-cm Petri plates containing 20 ml of 10% V8 juice agar were inoculated with an agar plug (5-mm diameter) obtained from a *P. sylvaticum* culture (COPS) grown on 10% V8 juice agar and then incubated at 25°C in the dark for 7 days. Nine corn seeds were placed on each plate, after which the plates were filled with 50 g of sterilized organic peat substrate. For the controls, seeds were placed on noninoculated plates of 10% V8 juice agar and filled with 50 g of sterilized organic peat substrate. Four replications were inoculated. Plates were maintained in a greenhouse at 23°C. After 14 days, similar symptoms to those observed in the field were present in the greenhouse, whereas control plants remained symptomless. *P. sylvaticum* (COPS) was reisolated from diseased roots as described above, thus confirming Koch's postulates. To our knowledge, this is the first report of *P. sylvaticum* on corn in China. This pathogen may pose a risk to corn production. The identification of the pathogen will help to develop effective strategies to control the disease.

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