Diseases Caused by Oomycetes

First Report of *Pythium ultimum* Causing Damping-Off of Sugar Beet (*Beta vulgaris*) in Montana, U.S.A.

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Sugar beet (*Beta vulgaris* L.) is a globally important crop for sugar. In May 2019, sugar beet seedlings were observed with wilting, lodging, and a few were dead in Glendive (46.970170°N, 104.838204°W), Montana. Symptoms appeared near the soil line as the stem (hypocotyl) turned dark brown to black with characteristic thread-like infections that resembled *Pythium* damping-off. It affected approximately 10% of the growing seedlings. Diseased sugar beet root tissues were excised with a sterile scalpel, and small pieces (10 mm²) were surface sterilized with 70% ethanol for 30 s, rinsed twice with autoclaved water, air dried, and transferred to potato dextrose agar medium amended with pimarcin-vancomycin-PCNB (Conway 1985). Four plates were incubated at 25°C in the dark (Masago et al. 1977), and 2 weeks later white, dense colony was observed (Zhang et al. 2018). The terminal smooth, globose oogonia (average 18.5 µm in diameter) and antheridia (average 14.5 × 9.5 µm) extended below the oogonium and were observed via a VWR N.A. 0.30 microscope. The morphological features of the four isolates were consistent with *Pythium ultimum* Trow (Watanabe 2002). Genomic DNA (Fungi DNA Isolation Kit 26200, Norgen Biotek Corp.) of four isolates was used for polymerase chain reaction (PCR) with the ITS6-ITS7 primers (Esmaili Taheri et al. 2017). Subsequently, PCR products were flushed by an E.Z.N.A Cycle Pure Kit (Omega), and four samples were sent for Sanger sequencing to GenScript (GenScript,

Piscataway, NJ). The sequences were identical and submitted to GenBank, NCBI (accession no. MN398593). The NCBI BLAST analysis showed 100% sequence homology to *P. ultimum* with the following GenBank accessions: KF181451.1, KF181449.1, and AY598657.2. A pathogenicity test was done on sugar beet with the same isolates in the greenhouse. Two-week-old *Pythium* culture was mixed with vermiculite and perlite mixer (Pro-Mix FLX) in the plastic trays (24" × 15" × 3") (22°C, 75% relative humidity). Sterile water (500 ml/tray) was added in the mixer to provide sufficient moisture. Twenty seeds of cultivar Hilleshog 4302 were sown in the tray, and the trays were replicated thrice with inoculated and mock treatments. Plants were watered as needed to maintain adequate soil moisture conducive for plant growth and disease development. Seven days after sowing, 50 and 100% germination was observed in the inoculated and control treatments, respectively. At the beginning of the second week, 30% postemergence damping-off was observed in the inoculated treatments. Diseased seedlings were gently pulled out from the pots, and similar symptoms were observed in the sugar beet seedlings as described previously. No incidence of disease was observed in mock-treated seedlings. Consistent reisolation of *P. ultimum* was morphologically and molecularly confirmed from the diseased seedlings, thus fulfilling Koch's postulates. *Pythium* spp. identification is prerequisite to develop effective management of pre- and postemergence damping-off. *P. ultimum* was previously reported in Nebraska to cause sugar beet seed rot and preemergence damping-off (Harveson 2006). To our knowledge, this is the first report of *P. ultimum* causing damping-off on sugar beet in the Sidney factory district in Montana.

References:

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