Diseases Caused by Oomycetes

First Report of *Pythium ultimum* Causing Crown Rot in Greenhouse-Grown *Cannabis sativa* in California

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Plant Dis. 105:1230, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2228-PDN>. Accepted for publication 17 December 2020.

In April of 2020 cuttings of *Cannabis sativa* (L.) in a greenhouse in San Mateo County, CA, were observed collapsing, and further observation revealed water-soaked stems, tan discoloration to the cortex, and discolored roots. The greenhouse irrigation system was supplied by a local stream. We collected 1-liter water samples from the intake pond, reservoir tank, irrigation lines, and local potable water tap. Water samples were filtered and plated as described previously (Rollins et al. 2016). Filter papers were removed after 24 h. Crown sections from four symptomatic plants and one asymptomatic plant were surfaced sterilized in 10% bleach for 5 min, rinsed in sterile deionized water, cut into 4-mm-long sections, plated onto V8 medium, and then incubated at room temperature for 3 days. White mycelial growth was observed from foci within the print of the filter paper from all irrigation water samples but not the potable water supply sample. Similar mycelial growth was observed from plated crown tissue from symptomatic plants only. Observation under a light microscope revealed characteristics congruent with *Pythium ultimum*, including aseptate hyphae and globose sporangia (Watanabe 2002). Mycelia was collected for DNA extraction from each of the water and plant sample plates, with DNA extractions performed using a Quick DNA Fungi/Bacterial Kit (Zymo Research Irvine, CA), and PCR amplified using primers ITS100/ITS4 as described by Riit et al. (2016). All amplicons were Sanger sequenced, aligned using SnapGene software (from GSL Biotech; available at

snappene.com), and compared with barcode reference sequences to identify the species using the BarCode of Life Database (BOLDsystems) within the National Center for Biotechnology Information nucleotide database. After trimming and aligning, all amplicons were found to be identical, yielding the 810-nucleotide-long consensus ITS amplicon (accession MW114807), which aligned with *P. ultimum* ITS sequences (e.g., accession HQ643886.1) with 100% identity and homology. We then completed Koch's postulates by using pure cultures from root sections of *P. ultimum* to stem inoculate *C. sativa* plants. We used a 3-mm corer to remove a disc of epidermis and applied a plug of pure culture to the wound. We inoculated 10 plants, with two plants mock-inoculated using clean V8 agar. Inoculation sites were wrapped in Parafilm, and plants were grown in the greenhouse for 20 days. Stems of mock- and oomycete-inoculated plants were examined for callus formation, and 30 cm of stem was excised from each plant. The mock-inoculated plants had fully callused inoculation sites and were discolored only where wounded. *P. ultimum*-inoculated plant inoculation sites were partially callused over and had tan discoloration of the cortex that extended 6.0 ± 2.0 mm above and below the inoculation site. Stem segments above and below inoculation sites were surface sterilized and plated on V8 medium as previously described and *P. ultimum* recovered from inoculated plants, confirmed as identical to the inoculum by ITS amplification and sequencing. Mock-inoculated plant stem cultures yielded no oomycete growth. Together, these results indicate that *P. ultimum* has the ability to cause crown rot in *C. sativa* in greenhouse cultivation.

References:

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The author(s) declare no conflict of interest.

Keywords: oomycetes, *Cannabis sativa*, pathogen detection, *Pythium ultimum*

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