Diseases Caused by Oomycetes

First Report of *Pythium myriotylum* Causing Root Rot in *Cannabis sativa* in California

T. L. Pitman,[†] R. N. Philbrook, and J. G. Warren

Dark Heart Nursery, Davis, CA 95618

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In June of 2020 we observed greenhouse-grown medical *Cannabis sativa* (L.) in peat-based soilless mixes in Sonoma and Monterey Counties, CA, showing stress symptoms (stunting, leaf chlorosis, and senescence), when moved to flower production conditions. We uprooted symptomatic and healthy plants and observed disease symptoms in symptomatic plants: reduced root mass, reduced root hair density, and necrotic lesions. Roots and soil samples were taken from infected and healthy plants for further analysis. Approximately 1 g of soil was added to 20.0 ml of deionized water, and 5.0 ml of the slurry was spread on water agar plates. Plates were rinsed after 24 h and then incubated for an additional 2 days. Aseptate hyphal growth was observed on all soil plates from symptomatic plant pots but not healthy plant pots. Four subcultures were transferred to V8 medium and grown for 3 days. Symptomatic and healthy roots were surface sterilized by soaking in 0.1% sodium hypochlorite for 5 min, rinsed in sterile deionized water, and 2-cm segments plated on V8 agar. After 48 h mycelial growth was observed from the cut ends of the lesion roots and not the asymptomatic roots. Four subcultures were transferred to V8 agar and grown for 3 days. DNA from all eight subcultures was purified using a Quick DNA Fungi/Bacterial Kit (Zymo Research, Irvine, CA) and then PCR amplified using ribosomal internal transcribed spacer (ITS) primers ITS100/ITS4 as described by Riit et al. (2016) and cytochrome oxidase (COX) primers OomCox-Levup/OomCox-Levlo as described by Robideau et al. (2011). All ITS and COX amplicons were Sanger sequenced and the resulting consensus sequences deposited in GenBank with accession numbers MW436422 (ITS) and MW448569 (COX). GenBank BLAST search identified the sequences as *Pythium myriotylum* ITS and COX sequences (e.g., AY598678.2 and HQ708745.1, respectively) with 100% coverage and 99.54% or greater identity. Cultures observed under light

microscope revealed morphological characteristics congruent with *P. myriotylum* including filamentous, inflated, and lobulate complexes of sporangia (diameter 5 to 16 µm), and oogonia with variable diameters of 22 to 29 µm and globose oogonia of 21 to 34 µm (Watanabe 2002). Zoospore soil drenches were used to prove pathogenicity using one root culture and one clean V8 as a control. Agar was cut into small pieces and added to 500-ml flasks of water. The flasks were incubated on a rotary shaker at 150 rpm at room temperature for 24 h. Motile zoospores were present in the water suspension at approximately 100 spores/ml from the *P. myriotylum* culture when observed under a light microscope. The zoospore suspension was then equally divided and applied to the soil of six *C. sativa* rooted cuttings in 1-gal pots. The control slurry was added to two *C. sativa* rooted cuttings. Plants were grown for 28 days with a 16-h photoperiod in a controlled environment and then observed for symptoms. Roots of all plants treated with zoospore suspensions had tan to brown lesions and reduced root hair density compared with plants treated with the control V8 agar suspension. Root samples from all eight plants were plated on V8 medium as previously described. After 48 h mycelial growth was observed from root sections from all zoospore-treated plants and not control plants. DNA extraction and PCR amplification for ITS and COX were performed on cultures for each plant, Sanger sequenced, and aligned with the previous sequences. All ITS and COX sequences were identical to the original sequences from greenhouse samples. *P. myriotylum* has previously been described infecting a wide range of host plants in temperate and tropical agriculture worldwide, greenhouse cannabis in Canada, and field hemp in the eastern United States (Bell 1973; McGehee et al. 2019; Perneel et al. 2006; Punja et al. 2019; Schuerger and Hammer 2009). This is the first report of *P. myriotylum* causing root rot in greenhouse-grown cannabis in California.

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[†]Indicates the corresponding author.

T. L. Pitman; tpitman@darkheartnursery.com