Diseases Caused by Oomycetes

First Report of Crown and Root Rot Caused by *Pythium aphanidermatum* on Industrial Hemp (*Cannabis sativa*) in Arizona

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During July and August 2020, symptoms of leaf yellowing and browning, sudden wilting, and death were observed on industrial hemp plants (*Cannabis sativa* L.) in several drip-irrigated fields in Yuma and Graham County, Arizona. About 85% of plants showed severe crown and root rot symptoms. A high percentage of affected plants collapsed under intensive heat stress. Shriveled stem tissue with necrotic lesions can often be seen at the base of the plant, extending upward more than 5 cm. Internal tissue of the main stem and branches was darkened or pinkish brown. Outer cortex of root bark was often completely rotten, exposing the white core. Cottony aerial mycelium was visible on the surface of stalk of some of the infected plants in two fields in Yuma. To identify the causal agent, a total of 20 symptomatic plants were collected from several fields across the state. Crown and root tissues from affected plants were harvested and rinsed in tap water to remove soils. Approximately 2- to 4-mm tissue fragments were excised from the margins of the affected stem and root lesions, surface sterilized in 0.6% sodium hypochlorite for 1 min, rinsed copiously in sterile distilled water, blotted dry, and plated on potato dextrose agar and on oomycete-selective clarified V8 medium containing pimarin, ampicillin, rifampicin, and pentachloronitrobenzene. Plates were incubated at room temperature for 2 days. Sixteen isolates were recovered, and their mycelial colonies resembled the morphology of *Pythium*. Based on the culture morphology on V8 medium, all isolates were tentatively identified as *Pythium aphanidermatum* with fast-growing, aseptate hyphae ranging from 3 to 7 µm in width, globose oogonia ranging from 25 to 31 µm in diameter, barrel-shaped antheridia, and globose oospores ranging from 15 to 21 µm in diameter (10 measurements) (Watanabe 2002). Genomic DNA was extracted from mycelial mats of three isolates using a

DNeasy Plant Pro Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The internal transcribed spacer region of rDNA was amplified with primers ITS1/ITS4, and three nucleotide sequences were obtained. All three sequences were identical and deposited under accession number MW380253 in GenBank. A BLASTn search revealed that MW380253 had a 100% query coverage and 100% match with sequences MK611609.1, KJ162355.1, and AY598622.2, obtained from isolates of *P. aphanidermatum*. To fulfill Koch's postulates, pathogenicity tests were conducted with two isolates using 12 seeds of a hemp line 14 sown in 12 1.9-liter pots filled with a steam-disinfested potting mix. Pots were placed in a plastic container and watered three times a week by flooding, to create waterlogged conditions. Plants were maintained in a greenhouse supplemented with artificial lighting of 14-h/10-h day/night light cycle. Plants were fertilized weekly with a 20-20-20 fertilizer at 1 mg/ml. Three weeks after sowing, four plants were inoculated with each isolate by drenching each plant with 200 ml of a 1×10^5 zoospore/ml suspension. Four plants, serving as a control, received each 200 ml of distilled water. Symptoms of leaf chlorosis, crown and root rot, and wilting were observed 3 weeks afterward, whereas control plants remained asymptomatic. *P. aphanidermatum* was reisolated from necrotic roots of inoculated plants but not from control plants. *P. aphanidermatum* was previously detected on industrial hemp in a research plot in Indiana (Beckerman et al. 2017) and is also known to affect other crops in Arizona during the summer months as well (Alcorn et al. 1984). This report is the first publication documenting *P. aphanidermatum* on field-grown hemp in Arizona. Industrial hemp (*C. sativa*) is an emerging crop in Arizona. The first plantings of hemp were in June of 2019, and 5,430 acres of hemp was planted in 13 counties in Arizona before the end of the year. The Arizona Department of Agriculture Industrial Hemp Program 2019 Year End Report confirms that nearly one-quarter of all hemp planted in 2019 did not receive a final state inspection due to crop loss. This disease is a potential constraint to hemp production in hot, arid climates, where copious water is used in combination with plastic mulch and/or drainage is poor.

References:

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