Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Black Root Rot (*Berkeleyomyces rouxiae*) on Greenhouse Hemp (*Cannabis sativa*) in Kentucky

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Plant Dis. 106:2534, 2022; published online as <https://doi.org/10.1094/PDIS-10-21-2146-PDN>. Accepted for publication 19 January 2022.

Hemp, *Cannabis sativa*, was reintroduced as a crop to the United States in 2014 after >80 years of prohibition. Since then, growers have focused primarily on cannabinoid production. Most medical/therapeutic hemp is asexually propagated from female plants in greenhouses and transplanted into fields. In December 2019, a commercial transplant producer in Fayette County, Kentucky, reported 30% dieback on 'Seagull' hemp. Grower-produced cuttings were potted into artificial media (unknown origin) immediately upon removal from the mist bench; these were intended to be sold to commercial producers within 4 to 6 weeks after repotting. The grower reported that symptoms of stunting, yellowing, and wilting developed ~2 weeks afterward on plants scattered across the greenhouse. Affected plants died within 1 week of symptom development. Brown to black lesions were distributed along roots that progressed into necrosis. Aleuriospores (chlamydospores) were visible with a dissecting microscope. Root pieces were surface sterilized with 10% household bleach for 2 min and plated onto cut centers of ~1-cm-thick sterilized carrot cross-sections (Williamson 2014). Resulting colonies were single-spored and maintained on PDA plates on laboratory benchtops (23°C, fluorescent lights). Three isolates were selected for pathogenicity testing and identification. Resulting colonies initially were white but darkened to black within 2 days following development of aleuriospores (chlamydospores). Aleuriospores were dark brown, formed in chains of four to five, and were 10.0 to 14.3 × 5.6 to 11.2 µm ($n = 50$). Endoconidia were hyaline, cylindrical, and 11.6 to 34.1 × 4.0 to 5.6 µm ($n = 50$). To confirm pathogenicity, soil was washed from three 4-week-old hemp plants, and roots were soaked in a 1×10^6 spore/ml conidial suspension for 5 min. Roots of three control plants were mock inoculated using sterile water. Plants were

transplanted into sterile potting media and maintained in separate trays. Inoculated plants developed leaf yellowing and wilting symptoms 14 days after inoculation (dai). Roots examined 21 dai had scattered brown lesions throughout and were colonized by the fungus. Aleuriospores and endoconidia isolated from symptomatic roots were identical to the inoculum. Controls did not develop symptoms by 21 dai, and no fungal growth resulted after attempts to culture. For molecular analyses, DNA was extracted from an actively growing (5 days) representative isolate (19FY005) on PDA using a Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA). Fragments of commonly used diagnostic loci (ITS, ACT, RPBII, and MCM7) were amplified as described by de Beer et al. (2014), Duong et al. (2012), and Fourie et al. (2015). Amplicons were sequenced by Sanger sequencing, and the consensus sequences were compared with the NCBI GenBank database by BLASTn. The ACT amplicon (OK135163) top hit was *Berkeleyomyces rouxiae* (MF967149.1) with 100% similarity, the MCM7 (OK135165) top hit was *B. rouxiae* (MF967103.1) with 100% similarity, the RPBII (OK135166) top hit was *B. rouxiae* (MF967194.1) with 99.80% similarity, and the ITS (OK135164) top hit was *B. rouxiae* (MF952402.1) with 99.82% similarity. *Berkeleyomyces* spp. (syn. *Thielaviopsis* spp.) is the causal agent of black root rot in field crops, such as cotton and tobacco, and in ornamentals, such as holly and pansy, and is common in greenhouse production systems. The description of *B. rouxiae* as a cryptic sister species of *B. basicola* indicates that reports of black root rot in many crops may be either *B. basicola* or *B. rouxiae*, as they are indistinguishable by morphology (Nel et al. 2018). Although both species have wide host ranges with no known host specificity, speciation may be important for pathogenicity, host susceptibility, and other cultural factors. As hemp acreage increases in the United States, black root rot may become more prominent in greenhouse transplant production systems and in fields.

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The author(s) declare no conflict of interest.

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Keywords: fungi, pathogen detection

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