Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium graminearum* Causing Flower Blight on Hemp (*Cannabis sativa*) in Kentucky

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In October of 2020, a grower in Boyle County, Kentucky, reported mold and blight symptoms on flowers of field-grown hemp. Plants were approaching harvest, and the mold was affecting 100% of the cultivar ‘White CBG’ being grown for cannabinoid (CBD) extraction. Mycelium colonized the flower heads and any seeds within bracts. Affected flower bracts were necrotic, and mycelium and necrosis in the most severe cases also encompassed adjacent (sugar) leaves. Necrotic symptomatic tissue was collected, disinfested in 10% bleach for 1 min, and cultured on acidified potato dextrose agar (PDA). Each isolate was single-spored, transferred to PDA, stored in 15% glycerol at -80°C , and maintained at room temperature under blacklight blue and fluorescent bulbs on a 12-h light-dark cycle. Colonies produced white-pink mycelia with a dark red pigment on the undersides. Conidia collected after 7 to 9 days were falcate and septate (five to six). No microconidia were produced. Macroconidia measured 35.4 to 49.7×3.4 to $5.8 \mu\text{m}$ ($n = 50$). The strains produced blue-black fertile perithecia on carrot agar when induced according to the method of Bowden and Leslie (1999). To confirm pathogenicity, flowers of hemp cultivars ‘Lifter’, ‘Trump Towers’, ‘Wife’, and ‘White CBG’ were inoculated in the greenhouse with a representative fungal strain (20Hemp010). Plants were inoculated at two different stages: when the styles were still green or after they had become senescent. Macroconidia were collected from 7- to 9-day-old cultures grown under a 12-h light-dark cycle. Plants were spray-inoculated with a 5×10^5 per ml conidial suspension in 0.05% Tween 20 until runoff. Flower heads were individually covered with clear plastic bags and incubated for 72 h at 95 to 100% humidity under greenhouse benches to avoid direct light. Bags were removed after 72 h, and plants were returned to the bench. Greenhouse conditions were 23 to 25°C with a 14-h photoperiod and 50% relative humidity. Symptoms developed 7 days after inoculation in 1% of the flowers inoculated when styles

were green and in 36% of the flowers that had senescent styles. Symptoms were similar to those initially noticed in Boyle County, including necrotic flower bracts and sugar leaves, and visible fungal growth. Symptoms were more severe on plants inoculated when styles were necrotic. Recovered fungi were morphologically similar to 20Hemp010. Genomic DNA was extracted from the mycelium with the Zymo Research Quick-DNA Fungal/Bacterial Miniprep Kit. A fragment of the translation elongation factor 1- α 1 gene was amplified with primers EF1 and EF2 as described by O'Donnell et al. (1998). Amplicons were sequenced, and the consensus (MZ407909) was compared with the NCBI GenBank Refseq database by BLASTn. The top hit was *Fusarium graminearum* with 100% identity (JF270185.1). Pairwise alignments via MycoBank *Fusarium* MLST and *Fusarium*-ID also revealed a top hit of *F. graminearum* with 100% identity (AY452957.1). Conidial and colony morphology were also consistent with *F. graminearum* (Leslie and Summerell 2006); thus, we conclude that this species was the causal agent of the flower blight and mold. The same disease was subsequently confirmed on hemp in Breathitt and Franklin Counties in Kentucky in 2020. This is the first report of this disease in Kentucky, although *F. graminearum* has been reported previously causing a similar flower blight on hemp in New York and North Carolina (Bergstrom et al. 2019; Thiessen et al. 2020). *F. graminearum* is common in Kentucky as a cause of *Fusarium* head blight on wheat and Gibberella ear rot on corn. In cereals, fungal infection is facilitated by the production of the mycotoxin deoxynivalenol (DON), which is harmful to humans and livestock (Desjardins and Hohn 1997). As hemp production in Kentucky continues to rise for production of CBD products and edible grains, accumulation and concentration of DON in these products could become a concern.

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