Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium falciforme* (FSSC 3 + 4) Causing Rot of Industrial Hemp (*Cannabis sativa*) in California

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Industrial hemp (*Cannabis sativa*) is a newly legal crop in California that is grown for cannabidiol oil, fiber, and seed. In August 2019, whole plant decline and root rot were observed affecting <5% of plants in two industrial fields in Fresno County, CA. Symptoms included chlorotic and collapsed foliage, stem vascular discoloration, and root rot with abundant mycelial growth. Stem and root segments (1 to 2 cm) from three to five diseased plants were agitated in 0.1% Tween 20 and soaked in 70% ethanol for 30 s and 1% sodium hypochlorite for 2 min. After incubating for 5 to 7 days on 1:10 potato dextrose agar (PDA) amended with tetracycline, *Fusarium* selective medium (FSM), and pimarcin + ampicillin + rifampicin + pentachloronitrobenzene (PARP) agar medium, white to pale cream aerial mycelium emerged from the tissue of all plants on PDA and FSM but not PARP. Isolates cultured on 0.1% potassium chloride agar formed heads of microconidia on long monophialides consistent with the *Fusarium solani* species complex (FSSC) (Leslie and Summerell 2006). To obtain pure cultures of two isolates (CS529 and CS530), a single hyphal tip was excised and grown on PDA. DNA was extracted from actively growing mycelium (PrepMan Ultra kit). The translation elongation factor gene (EF-1 α) was amplified via PCR using EF1/EF2 primers (O'Donnell et al. 1998). Sequences of the two isolates were identical and deposited under accession number MW892973 in GenBank. The 599-bp sequence was 99.33% identical to FSSC 3 + 4 (*Fusarium falciforme*) accessions FD_01443_EF-1a based on *FUSARIUM*-ID BLAST analysis. To evaluate pathogenicity, stems of hemp plants (cv. Berry Blossom; $n = 8$ plants per isolate) were wounded by penetrating

the epidermis in an area about 0.5 cm square by 1 mm deep and 8 inches above the soil line. A 0.5-cm-diameter plug of 7-day-old *F. falciforme*-colonized PDA was placed against the wound. Inoculation sites were loosely wrapped with Parafilm for 2 days. A negative control consisted of a sterile PDA plug ($n = 3$). Treatments were arranged in a completely randomized design in a greenhouse. The experiment was conducted once, due to regulatory restrictions at campus facilities. At 61 days postinoculation, external stem lesions were significantly larger in diameter ($P < 0.05$; Tukey's HSD) in plants inoculated with CS529 (8 ± 1 mm) compared with the control (2 ± 0 mm) and larger but not significant for CS530 (6 ± 1 mm). Internal stem lesions (i.e., rot in stele) were observed in plants inoculated with CS529 (9 ± 3 mm); stem rot was very minor in plants treated with CS530 (1 ± 1 mm) and nonexistent for control plants. No other disease symptoms were observed. *F. falciforme* was isolated from stems of CS529- and CS530-inoculated plants. Sequences of reisolates matched 100% with accession MW892973. These results suggest that *F. falciforme* causes rot in hemp in California. These studies specifically confirm stem rot abilities; field observations of root rot indicate root rotting abilities, but further tests are needed for confirmation. This is the first report of *F. falciforme* causing disease in industrial hemp. FSSC was described as causing foot rot in hemp in Italy (Sorrentino et al. 2019), but these isolates belonged to phylogenetic species 5 (*F. solani*) not *F. falciforme*. In addition, *F. falciforme* was reported as causing root rot in hydroponically grown cannabis (Punja and Rodriguez 2018). These studies provide the foundation for development of management tools for hemp disease.

References:

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