Diseases Caused by Fungi and Fungus-Like Organisms

First Report of White Root Rot of Hemp (*Cannabis sativa*) Caused by *Dematophora necatrix* in Campania Region (Southern Italy)

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Industrial hemp (*Cannabis sativa* L.) was cultivated in Italy until the end of the Second World War. Since then, it has been abandoned and substituted with other crops mainly due to legal restrictions and public concerns. Public legislation passed in 2016 has allowed for the production of hemp seeds, flowers, and fibers (law no. 242/2016). During a 2019 survey on hemp sanitary status in the province of Naples (40°57.96012'N, 14°22.937056'E), 'Kompoly' plants with symptoms of root rot were observed at a private farm and collected for further analysis at the phytosanitary laboratory of CREA in Caserta. Death generally occurred within 2 to 3 weeks after the appearance of the first symptoms, occurring on ~10% of plants, consisting of yellowing, canopy wilt, and signs of roots covered with white mycelium and fan-like mycelium under the bark. The causal agent was isolated from small root segments that were excised from symptomatic plants; the surface was disinfected with 2% sodium hypochlorite, placed on potato dextrose agar (PDA) amended with streptomycin sulfate (100 mg/liter), and incubated in the dark at 25°C for 5 days. Small pieces (2 to 3 mm) at the edge of the resulting colonies were subcultured onto PDA and incubated at 25°C in the dark for 1 week. The mycelia from 15 isolates showed pear-shaped swellings adjacent to the septa. The conidia were aseptate, hyaline, ellipsoid to ovoid, and 3 to 5 × 2.5 to 3 µm. Based on the morphological characteristics, the fungus was identified as *Rosellinia necatrix* Berl. ex Prill. (Singleton et al. 1992), a fungus taxonomically revised to *Dematophora necatrix* R. Hartig (Wittstein et al. 2020). To confirm this finding, total DNA was extracted from five isolates using a

DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and ITS spacer PCR amplified with primers ITS1-ITS4 (White et al. 1990). The size-expected amplicons of 536 bp were purified and sequenced, and the resulting sequence was trimmed and deposited in GenBank under the accession number MK937913. BLASTn analysis revealed 98.83% nucleotide identity with some representative isolates of *D. necatrix* (MK888684.1; KT343972.1). To fulfill Koch's postulates, pathogenicity tests were carried out on 15 Kompoly potted hemp plants (4 weeks old). The inoculation was performed by adding 3 g of millet seeds inoculated with 10 mycelial plugs, taken from the margins of a *D. necatrix* actively growing colony, per liter of sterile peat and perlite substrate in single pots. Moreover, 10 hemp plants were inoculated with sterilized millet seed and served as negative controls. All plants were incubated at 25°C. After 3 weeks, inoculated plants exhibited foliar chlorosis and leaves apical wilting, and died in 2 weeks, similar to what was observed in the field. Control plants did not show any symptoms. The fungus was isolated from the roots in all 15 inoculated plants and confirmed to be *D. necatrix* based on morphological and molecular analysis, carried out with a second primer pair EF1-983F/EF1-2218R targeting the transcription elongation factor 1-α (Rehner and Buckley 2005) (MW541068) that showed 99.67% nt in BLASTn analysis. To our knowledge, this is the first report of *D. necatrix* infecting hemp in Europe. The farm where the problem arose has a history of cultivation for the production of apples for over 30 years. Therefore, an adaptation of *D. necatrix* to the new host is hypothesized. An in-depth knowledge on the diseases of hemp will be needed to relaunch hemp cultivation in this area.

References:

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