Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Mycoleptodiscus terrestris* Causing Root Rot of Soybean in Indiana

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During the summers in 2019 and 2020, 137 soybean (*Glycine max* [L.] Merr.) seedlings (V1 to V3 stage) showing stunting, delayed emergence, and/or crown lesions were collected at Purdue's Agronomy Center for Research and Education in West Lafayette, Indiana. Four seedlings were stunted with reddish-brown girdled lesions along the hypocotyl and crown, rotted tap and lateral roots, and brown discoloration of the cortex and vascular tissues. Four fungal isolates (AC4, AC58, AC96, and AC127) were recovered by plating surface-sterilized symptomatic root tissue onto water agar plates and incubating on the benchtop until mycelia emerged. The growing hyphal tips were transferred to the semiselective medium DCPA (Andrews and Pitt 1986). On potato dextrose agar, the fungal colonies developed olivaceous green mycelia, which melanized into a mat of black microsclerotia with time, and no conidia were observed. On 1.5% water agar plates amended with twice-autoclaved soybean leaf and root tissue collected from flowering soybean plants, conidia were formed in sporodochia in darkness at 28°C within 1 week. Conidia were one to two septate, cylindrical with two setae on either end, and measured 20.8 to 26.4 × 4 to 5.6 µm (average 23.9 × 4.7 µm, $n = 20$). The morphological characters matched with the description of *Mycoleptodiscus terrestris* (Gerd.) Ostaz. (Gerdemann 1953). Species identification was further confirmed by sequencing the internal transcribed spacers (ITS) region of rDNA amplified by ITS1 and ITS4 primers (White et al. 1990) and the translation elongation factor 1 alpha (TEF1-α) gene using 983F and 1567R primers with annealing temperature at 53°C (Rehner and Buckley 2005). The sequences were deposited in GenBank under the

following accession numbers: MW002684, MT998441, MW010258, and MW010260 (ITS); and MW015941 to MW015944 (TEF1-α). The GenBank BLAST searches revealed 100% identity in the ITS region (accession NR_145373.1) and 99.75% identity in the TEF1-α region (MK495977.1) to *M. terrestris*. Pathogenicity testing was conducted on soybean seedlings (cv. Williams) at V1 growth stage using a root-dip assay. Isolate AC58 was grown in a modified cotton seed meal broth (CSMB) to produce microsclerotia as inoculum (Gray 1978; Shearer and Jackson 2006). Microsclerotia concentration was measured using a hemocytometer and adjusted to 1.5×10^4 /ml. Five soybean seedlings each were dipped into inoculum or sterile CSMB for 30 min and then planted individually in vermiculite-filled Styrofoam cups placed on flooded trays in 16-h photoperiod light racks at room temperature. Seven days after inoculation, all inoculated plants were visibly stunted with root and crown symptoms identical to field symptoms, whereas all controls were healthy. *M. terrestris* was successfully reisolated from inoculated plants, but not from the controls, and identified by morphology and sequencing as above. *M. terrestris* has been previously reported causing root rot of soybean in Illinois (Gray 1978) and Wisconsin (Smith et al. 1998). To our knowledge, this is the first report of *M. terrestris* infecting soybean in Indiana. Increased geographic distribution of this pathogen warrants more attention for its control. *M. terrestris* has been proposed as a biological control agent against multiple aquatic weeds (Shearer and Jackson 2006; Verma and Charudattan 1993). Introduction of this fungus into soybean production regions should be avoided.

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