Disease Notes

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Mycoleptodiscus terrestris* Causing Crown and Root Rot of Alfalfa (*Medicago sativa*) in Minnesota

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Mycoleptodiscus terrestris (Gerd.) Ostaz. has been studied extensively as a potential mycoherbicide against aquatic weeds since the early 1970s (Mathur and Gehlot 2018; Shearer 1998). However, it is also a pathogen on many legumes including alfalfa (Gerdemann 1953; Smith et al. 1998). In August 2019, alfalfa plants with stunted yellow foliage and rotted stems at the base of the crown were observed in a variety trial planted in spring 2018 at the University of Minnesota, St. Paul, Minnesota. Affected plants were in patches across the field with approximately 20% of plants showing symptoms. Plants from patches had few lateral and fibrous roots, and dark lesions appeared in the crown and root tissue. Tissue samples (5 mm^2) from 10 plants with symptomatic crown and root tissues were surface disinfested with 70% ethanol for 5 min, followed by 30 s in 10% NaOCl, and then rinsed three times in sterile distilled water. Samples were air dried and placed on water agar amended with 25 µg/ml of rifampicin. Plates were incubated at room temperature for 3 days, and fungal hyphal tips from tissue samples were transferred to potato dextrose agar (PDA). After 7 days, isolates were tentatively identified by morphological characteristics as *M. terrestris* (Ostazeski 1967). The hyaline mycelia turned from olive-gray to dark gray with age. Abundant dark microsclerotia formed 5 days after incubation that varied in size and shape, measuring 300 to 860 × 270 to 600 µm. Molecular identification of two representative isolates (DAZ5 and DAZ9) was done by sequencing the internal transcribed spacer (ITS) regions of the rDNA, the translation elongation factor 1α (TEF1), and the second largest subunit of RNA polymerase II (RPB2) genes. For both isolates the ITS, TEF1, and RPB2 genes were amplified and sequenced with primers ITS1/4 (White et al. 1990), 983F/1567R (Rehner and Buckley 2005), and fRPB2-7cR/RPB-5F2 (Liu et al. 1999; Sung et al. 2007), respectively. ITS amplification conditions followed White et al. (1990); the TEF and the partial RPB2 gene were obtained by using a touchdown PCR protocol as described in Rehner and Buckley (2005) and Woudenberg et al. (2017), respectively. Based on a BLAST search of the NCBI nucleotide database, the ITS sequences (GenBank MN851265.1 and MN851266.1) had 100% identity with *M. terrestris* strain CBS 231.53 (MK487754.1). The TEF1 (MN873019, MN873020) and RPB2 sequences (MN873021, MN873022) had 100% identity with *M. terrestris* strain IMI 159038 (MK495977.1, MK492735.1). A pathogenicity test was performed by inoculating five 3-week-old alfalfa plants per cultivar (cvs. DKA44-16RR, Vernal, 53V52, and Agate) with isolates DAZ5 and DAZ9. Plants were inoculated around the exposed stem base with three 5-mm-diameter PDA plugs from a culture of *M. terrestris* covered with microsclerotia. Control plants were inoculated with sterile PDA plugs. After inoculation plants were incubated at 25°C with a 16-h photoperiod in a growth chamber. The first symptoms appeared 2 months after inoculation on all cultivars as a dark lesion at the stem base followed by yellowing and rotting of stems. Inoculated plants had more fibrous roots than the controls. The control plants were symptomless with healthy root development. The pathogen was reisolated from all infected alfalfa cultivars and had the same morphology as isolates DAZ5 and DAZ9, fulfilling Koch's postulates. To our knowledge, this is the first report of *M. terrestris* causing crown and root rot disease of alfalfa in Minnesota.

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