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DISEASE NOTES

First Report of Fusarium Wilt of Alfalfa Caused by *Fusarium oxysporum* f. sp. *medicaginis* in Wisconsin

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Fusarium wilt is an economically important vascular disease of alfalfa (*Medicago sativa*) throughout the world (Rhodes 2015). During the summers of 2013 to 2016, alfalfa plants with foliar wilt symptoms and reddish-brown discoloration in the root stele and basal stem, consistent with symptoms of Fusarium wilt (Rhodes 2015), were observed across disease nursery field plots with multiple germplasms near Arlington, Wisconsin. Symptomatic roots were dipped in 70% ethanol, flame sterilized, cut into thin slices, and incubated on potato dextrose agar medium amended with 0.05% streptomycin. Colonies of white mycelia with tan sporodochia producing macro- and microconidia morphologically similar to *Fusarium* spp. were observed after 3 weeks of incubation at room temperature. Six single-spore isolates were characterized using spore morphology, diagnostic DNA sequences, and a pathogenicity assay. Macroconidia were hyaline, falcate, had three to five septa, and measured 25 to 45 × 6 µm. Microconidia were hyaline, oval, nonseptate, and measured 9 × 3 µm. The rDNA internal transcribed spacer (ITS) region and translation elongation factor 1-α (TEF) were PCR amplified, sequenced, and used for polyphasic identification (http://www.cbs.knaw.nl/fusarium/). Best matches at 99.78% similarity were to the *F. oxysporum* species complex. The ITS and TEF sequences of a representative strain, FW16B,

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were deposited in GenBank under accession numbers MF435930 and MF442438, respectively. Pathogenicity was tested with four replications of 50 plants per treatment according to a standardized protocol (Brummer and Nygaard 1995). Agar plugs from the six isolates were incubated in potato dextrose broth on a shaker for 7 days to produce microconidia. Roots of 8-week-old plants of the Fusarium wilt–susceptible cultivar MNGN-1 and of resistant cultivar Agate were washed, clipped to 10 cm, and soaked in a spore suspension (1×10^6 microconidia/ml) overnight at 11°C and planted in a soil mix in pots the next day. Roots of mock-inoculated plants were clipped and soaked in water. Plants were grown in a greenhouse with 16 h of light and 8 h of darkness at 18 to 32°C. After 12 weeks, roots were cross-sectioned and rated for disease symptoms as described previously (Brummer and Nygaard 1995). Resistant plants had symptomless roots or discrete dark specks in the stele, whereas susceptible plants had dark discoloration in an arc or ring pattern in the stele, had severe necrosis of the entire root, or plants were dead. Disease resistance was 3% for the susceptible cultivar and 39% for the resistant cultivar, which is consistent with the range expected for these check cultivars (Brummer and Nygaard 1995). *Fusarium* was reisolated from symptomatic roots, completing Koch's postulates. Mock-inoculated plants had few disease symptoms, and the percentage of resistant plants was significantly different from inoculated plants for both varieties ($P < 0.0001$). The six strains were submitted to the University of Minnesota Mycological Culture Collection under accessions FW13B, FW13F, FW14A, FW14D, FW16A, and FW16B. These results suggest that *F. oxysporum* f. sp. *medicaginis* was isolated from the diseased alfalfa plants. Continued improvement of varieties would be aided by recognition of Fusarium wilt in Wisconsin, a major alfalfa-producing state and an important site for alfalfa research and breeding. Changing climate conditions or reduced vigilance of breeding efforts could lead to this disease becoming a threat to alfalfa production.

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