

Fusarium wilt of alfalfa caused by *Fusarium oxysporum* f. sp. *medicaginis* identified in Wisconsin

Jennifer Peterson, S&W Seed Company

Deborah Samac, USDA-ARS

Craig Grau, University of Wisconsin

Alfalfa is an important forage crop in Wisconsin. Fusarium wilt of alfalfa was first described in the United States in 1927 but has not been officially recognized in Wisconsin until recently. 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, were observed near Arlington, Wisconsin. Six isolates were obtained from symptomatic roots, and characterized using spore morphology, diagnostic DNA sequences, and a pathology 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, were deposited in GenBank under accession numbers MF435930 and MF442438, respectively. Pathogenicity was tested with five replications of 50 plants per treatment according to a standardized protocol (Brummer and Nygaard 1995). Roots of 8-week-old plants of the Fusarium wilt-susceptible cultivar MNGN-1 and of resistant cultivar Agate were clipped and inoculated in a microconidial suspension produced from the six isolates (1×10^6 microconidia/ml). Roots of mock-inoculated plants were clipped and soaked in water. Plants were incubated in a greenhouse. 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 cultivars ($P < 0.0001$). 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. Changing climate conditions or reduced vigilance of breeding efforts could lead to this disease becoming a threat to alfalfa production.