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

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Introduction

Alfalfa (*Medicago sativa*) is an important forage crop in Wisconsin, being the third most important crop for the Wisconsin economy in 2017 and representing more than 10% of alfalfa acres planted in the United States (3).
Fusarium wilt of alfalfa was first described in the United States in 1927, and is an important alfalfa disease throughout the world (2). During the summers of 2013 to 2016, alfalfa plants with foliar wilt symptoms were observed across disease nursery field plots with multiple germplasms near Arlington, WI. Digging these plants revealed reddish-brown discoloration in the root stele and basal stem, consistent with symptoms of Fusarium wilt.
Six single-spore isolates were characterized using spore morphology, diagnostic DNA sequences, and a pathogenicity assay.

Figure 1. Alfalfa field with Fusarium wilt symptoms.

Materials and methods

Isolation of Fusarium: 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. Once fungi resembling Fusarium were observed, hyphal tips were removed and grown individually on new agar plates. From these cultures, spores were transferred to new plates and cultures were evaluated for morphology, DNA sequence, and pathogenicity.

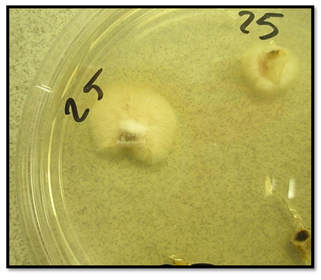

Figure 2. Alfalfa root sections on PDA.

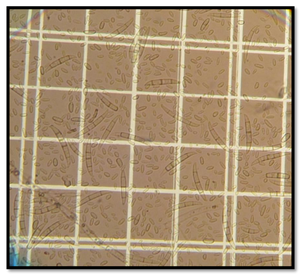

Figure 3. Fusarium macro and microconidia.

Results

Morphology

Morphology was consistent with *Fusarium oxysporum*. Colonies of white mycelia with tan sporodochia producing macro- and micro-conidia morphologically similar to *Fusarium* spp. were observed after 3 weeks of incubation at room temperature. 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.

Results

Pathogenicity test

Pathogenicity was tested with five replications of 50 plants per treatment according to a standardized protocol (1). 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.

Figure 4. Inoculated alfalfa plants with varying Fusarium wilt susceptibility.

After 12 weeks, roots were cross-sectioned and rated for disease symptoms as described previously (1). 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. Fusarium was re-isolated from symptomatic roots, completing Koch's postulates.

Table 1. Expected resistance of cultivars (1) and experiment mean

	Approximate Expected resistance*	Acceptable Range	Experimental average
Resistant Agate	45%	35-55%	39%
Susceptible MNGN-1	4%	0-8%	3%

*Controlled environmental evaluations can be more severe than field evaluations.

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 (1).

Table 2. Randomized complete block variance analysis of Fusarium resistance between cultivars

Source	DF	SS	MS	F	P
rep	4	87.2	21.81	0.47	0.7540
cultivar	5	11014.4	2202.88	48.11	0.0000
Error	20	915.7	45.79		
Total	29				

mean 28.89 CV 23.4

Mock-inoculated plants had few disease symptoms, and the percentage of resistant plants was significantly different from inoculated plants for both cultivars ($P < 0.0001$).

Table 3. Variance analysis of inoculated and uninoculated cultivars

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cultivar	1	6469.5	6469.48	152.31	0.0000
inoculated	1	3071.9	3071.89	72.32	0.0000
cultivar*inoculated	1	66.2	66.22	1.56	0.2230
Error	26	1104.4	42.48		
Total	29	12019.8			

Model Summary S = 6.52 R-sq= 90.81%

Results

Diagnostic DNA Sequencing

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.

Figure 5. Healthy alfalfa root compared with root expressing Fusarium wilt symptoms. The left image is of plants incubated in a greenhouse, the right image is of plants dug from the field.

Conclusion

These results suggest that *F. oxysporum* f. sp. *medicaginis* was isolated from the diseased alfalfa plants. The six strains used in the study were submitted to the University of Minnesota Mycological Culture Collection under accessions FW13B, FW13F, FW14A, FW14D, FW16A, and FW16B.
Fusarium wilt of alfalfa is more severe in warm areas with high soil temperatures, and despite a long time presence in Wisconsin, has not been officially recognized until recently. Increased volatility in weather patterns, and increasing summertime temperatures would indicate a need for alfalfa breeders to be proactive in developing resistance to this disease in areas that are not traditionally heavily impacted by this wilt disease. Identification and recognition of this pathogen in Wisconsin will help aid breeding efforts in screening for this disease.

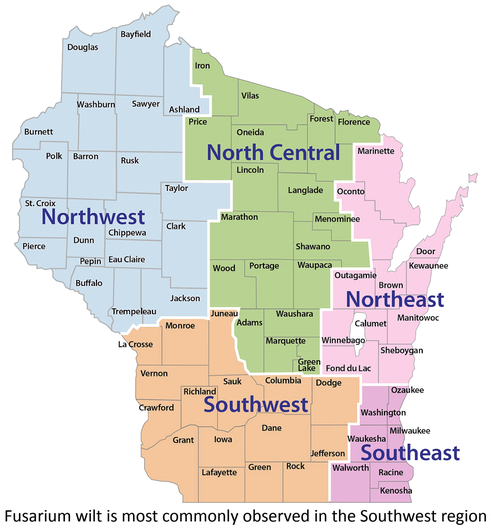

Figure 6. Fusarium wilt is most commonly observed in the Southwest region of Wisconsin.

References:

1. Brummer, J. E., and Nygaard, S. L. 1995. Fusarium Wilt Resistance - Greenhouse/Controlled Environment. Page D-6a in: Standard Tests to Characterize Alfalfa Cultivars. 3rd ed. North American Alfalfa Improvement Conference. https://www.naaic.org/stdtests/fusariumwilt2.html

2. Rhodes, L. H. 2015. Fusarium Wilt. Pages 41-42 in: Compendium of Alfalfa Diseases and Pests. 3rd ed. D. A. Samac, L. H. Rhodes, and W. O. Lamp, eds. American Phytopathological Society Press, St. Paul, MN.

3. USDA National Agricultural Statistics service. https://www.nass.usda.gov.