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Disease Notes

# First Report of *Stemphylium globuliferum* Causing Stemphylium Leaf Spot on Alfalfa (*Medicago sativa*) in the United States

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Stemphylium leaf spot occurs in most areas where alfalfa (*Medicago sativa*) is grown. In the United States, *Stemphylium botryosum* is reported to be the predominant pathogen (1), although *S. vesicarium* and *S. herbarum* are also observed. *S. alfalfae* was isolated on alfalfa in Australia (4) and *S. globuliferum* was reported in Egypt and Korea. In April and May 2012, alfalfa plants with leaf spot symptoms were observed in Rosemount and Waseca, MN, and in Arlington, Tomah, and Waupaca, WI. Initial symptoms consisted of white to tan spots with a brown border, 2 to 3 mm in diameter, circular to oval, enlarging to 5 to 8 mm in diameter. Large lesions often coalesced. Small, narrow, brown lesions occurred on petioles. Lower killed leaves remained attached to the primary stem. Spots were larger than those caused by the cool temperature biotype of *S. botryosum*. Conidia formed on lesions after 48 h in a moist chamber. Conidia were removed with a fine glass rod, germinated on 1% water agar, and single hyphae transferred to V8 agar (V8A). After 2 weeks under room light, plates were placed under UV light to stimulate spore production. Conidia on host material were borne singly on straight, unbranched, smooth conidiophores, medium brown at the apex. Conidia were medium to dark brown with small papillae, subspherical with 3 to 4 transverse and 3 to 4 complete or near complete longitudinal septa, with a distinct constriction at the median

transverse septum. Conidia were 27.5 to 32.5 µm long × 20 to 22.5 µm wide with a length/width (L/W) ratio of 1.2 to 1.5. Conidia on V8A were smaller, 25 to 30 µm long × 12.5 to 19 µm wide with a L/W of 1.6 to 1.8. Ascostromata 300 µm in diameter formed on leaves held at 4°C for 2 months as well as on culture plates after 1 month. Ascospores from leaves were golden brown to reddish, 40 to 42.5 × 20 µm, slightly broader in the upper half of the spore, with 7 to 8 transverse septa and one complete longitudinal septum with several incomplete septa. Ascospores from culture were smaller, 27.5 to 30 × 12.5 to 15 µm wide. These morphological features are consistent with the description for *S. globuliferum* (3). DNA was extracted from pure cultures of SAr301 and SWp202, isolated from plants grown in Arlington and Waupaca, respectively, and used to amplify ITS1-5.8S-ITS2 rDNA using primers ITS1 and ITS4, *GPD* with primers GPD1 and GPD2, *EF-1α* with EF446f and EF1473R, and the intergenic spacer between *vmaA* and *vpsA* with primers ATPF2 and GTP604R (2). In sequence comparisons made by BLASTn searches of GenBank, the ITS (KF479193), *GPD* (KF479194), and *EF-1α* (KF479195) sequences from *S. globuliferum* were different from the gene sequences of *S. botryosum* but identical to those from *S. vesicarium*, *S. herbarum*, and *S. alfalfae*. The *vmaA-vpsA* spacer sequence (KF479196) of *S. globuliferum* had 3 nucleotide differences from *S. vesicarium* and *S. herbarum* and 4 nucleotide differences from *S. alfalfae*, demonstrating that this sequence is useful for species discrimination. Conidia from strains SAr301 and SWp 202 were suspended at $10^4$/ml in sterile water with 0.01% Tween 20 and used to inoculate 12 alfalfa plants using a handheld sprayer. Plants were kept at 100% RH for 48 h, then grown at 20°C with a 16-h photoperiod. After 2 weeks, lesions similar to those seen in the field were observed on leaves of all plants. Symptomatic leaves placed in moist chambers produced conidia with the size and morphology of *S. globuliferum* within 48 h. This is the first report to our knowledge of *S. globuliferum* causing disease on alfalfa in the United States. Cultures were deposited in the University of Minnesota Mycological Culture Collection.

*References*: (1) W. A. Cowling et al. Phytopathology 71:679, 1981. (2) P. Inderbitzin et al. Mycologia 101:320, 2009. (3) E. G. Simmons. Mycologia 61:1, 1969. (4) E. G. Simmons. Sydowia 38:284, 1985.



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