Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stagonosporopsis heliopsidis* Causing a Leaf Spot on Whorled Sunflower, *Helianthus verticillatus*, in the United States

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Whorled sunflower, *Helianthus verticillatus* Small (Asteraceae), is a federally endangered species (U.S. Fish and Wildlife Service 2014) growing in only a few locations in Tennessee, Georgia, Mississippi, and Alabama (Ellis et al. 2008; Matthews et al. 2002) and recently in Virginia (Edward Schilling, *personal communication*) in the United States. This herbaceous perennial produces yellow, daisy-like inflorescences from September through October and is of interest for use in landscapes because the flowers attract numerous native bees and other insects (Edwards et al. 2020; Strange et al. 2020; Trigiano et al. 2021). In May 2021, black necrotic spots surrounded by chlorotic halos appeared on various locations on the blade of mature leaves on landscape plants in Knoxville, TN. Symptomatic leaves were washed in running water for 10 min, surface-sterilized with 1.25% NaOCl for 8 min, and rinsed with sterile, distilled water. Lesions were excised with some healthy tissue, cultured on potato dextrose agar (PDA) augmented with 10 mg/liter of rifampicin, and incubated at room temperature. White to dark-brown hyphae grew from the lesions and were transferred to fresh medium until pure cultures were obtained. Cream-colored and slightly pinkish spore masses were evident after 10 weeks of culture. Conidia were cylindrical and ellipsoidal to subglobose at one end. The mean dimensions of conidia were 5.4 µm (3.9 to 6.8 µm) in length and 2 µm (1.7 to 2.5 µm) in width ($n = 20$). Conidia matched the description of *Stagonosporopsis heliopsidis* (formally *Phoma heliopsidis* and *Phyllosticta heliopsidis*) (Chen et al. 2015). The morphology of the conidia from *S. heliopsidis* differs slightly from the globose conidia of

its closely related sister species, *S. helianthi* (Chen et al. 2015). PCR was performed with ITS1 and ITS4 primers (White et al. 1990) using the Phire Direct Plant PCR kit (ThermoFisher Scientific, Waltham, MA) and sequenced at a commercial sequencing center. The sequences were concatenated into one consensus and identified as *S. heliopsidis* with a 100% match to GenBank number GU237924.1. A sequence was deposited in GenBank with accession number OK315471. Koch's postulates were completed using detached leaf assays because *H. verticillatus* is an endangered species and only a few plants were available. Five surface-sterilized, symptomless, sign-free leaves were dissected bilaterally, and halves were placed individually on 1.5% water agar supplemented with 10 mg of rifampicin/liter. Mycelial plugs grown on PDA were positioned in contact with one half-leaf, and the remaining half-leaf was treated with a sterile PDA plug as a control. Cultures were incubated for 7 days at room temperature in ambient light. All control halves of the leaves remained green and healthy, whereas the inoculated leaves developed lesions with the same appearance as lesions on the whole plant. The pathogen was reisolated using the protocol described previously and sequenced (OK315470). This fungus causes very limited aesthetic damage to leaves but does not affect the growth and flowering of ornamental plantings of *H. verticillatus*. To our knowledge, this is the first report of *S. heliopsidis* on *H. verticillatus* in the United States.

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