Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Alternaria alternata* on *Sonchus asper* in China

J. X. Zhang,¹ Y. Kang,² T. T. Xu,¹ X. Y. Xu,¹ C. X. Li,¹ K. F. Chen,¹ and H. Q. Cao^{1,†}

¹ College of Plant Protection, Anhui Agricultural University, Hefei 230036, China

² Department of Chemical and Chemical Engineering, Hefei Normal University, Hefei, 230601, China

Funding: This research was supported by National Natural Science Foundation of China (31901902) and Natural Science Research Projects of Colleges and Universities in Anhui Province (KJ2018A0146). Plant Dis. 105:503, 2021; published online as <https://doi.org/10.1094/PDIS-05-19-0940-PDN>. Accepted for publication 29 August 2020.

Sonchus asper (spiny sowthistle), belonging to the Asteraceae, is a problematic weed in grain crops, orchards, and turf (Cho et al. 2019). In April 2016, about 90% of the *S. asper* plants infesting a pear orchard had symptoms with black spots densely distributed on the leaves and stems in Da Yang Town, Luyang District, Hefei, Anhui (N 31°55'43", E 117°11'40"). The foliar lesions were mostly circular (3.0 to 1.5 cm in diameter) or irregular in shape; lesions on the stem were elliptical to irregular in shape, running along the vascular bundle and about 3 to 6 mm long × 2 to 3 mm wide. Severely affected plants had their leaves become completely blighted, and eventually the plants died. Fifteen tissue pieces from five symptomatic leaves and stems were surface sterilized with 0.5% NaClO for 2 min, rinsed three times with sterile water, and then plated on quarter-strength potato dextrose agar (PDA) medium. Fourteen fungal isolates were obtained from the tissues, and the isolation frequency from the plant tissue pieces was almost 93%. Fungal colonies were circular, initially white, and eventually turned to dark olive or black along with profuse sporulation. The conidia were tawny to brownish green, obclavate to obpyriform, with a cylindrical or coniform short beak, ranging from 16.8 to 39.8 µm long × 6.3 to 14.7 µm wide with two to five transverse and zero to two longitudinal septa ($n = 50$) and were borne on branched conidiophores. These morphological characteristics were similar to those described for *Alternaria alternata* (Simmons 2007). For one representative isolate, the internal transcribed spacer (ITS) region of rDNA, partial coding sequence of the actin (ACT) gene, partial Alt a 1 major allergen (ALT) gene, and

partial glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene were amplified and sequenced with primers ITS1/ITS4 (White et al. 1990), ACT512F/ACT783R (Carbone and Kohn 1999), Alt-4for/Alt-4rev, and gpd1/gpd2 (Lawrence et al. 2013), respectively. Sequences were submitted to GenBank (ITS, MH886523; ACT, MH892480; ALT, MN655781; GAPDH, MN655782). Based on a BLAST analysis, ITS, ACT gene, and GAPDH gene had 99.8% to 100% identity with the existing sequences of the ex-type CBS 916.96 of *A. alternata* (Fries) Keissler (ITS, AF347031; ACT, JQ671702; GAPDH, AY278808, respectively), and ALT gene showed 100% identity with the *A. alternata* isolate SCWC10 (MG199093). Thus, the pathogen was identified as *A. alternata* based on its morphological and molecular characteristics. Pathogenicity tests were conducted on six- to seven-leaf-stage potted *S. asper* plants (one plant per pot). Five-millimeter-diameter fungal disks, which were excised from the edge of a 3-day-old *A. alternata* colony, were placed on healthy leaves of five plants. Sterile PDA disks of the same size were used as controls. In addition, a conidial suspension (10^7 conidia/ml) was also inoculated on healthy leaves of five plants, and sterile distilled water was used as a control. Both inoculation experiments were conducted in the greenhouse at $25 \pm 2^\circ\text{C}$, 90% relative humidity, 12-h light/dark photoperiod, and repeated at least three times. Lesions were observed on all the leaves within 12 h after fungal disk inoculation. All pathogen-inoculated plants developed lesions, similar to those observed on the symptomatic leaves in the field 12 days after conidial inoculation. No symptoms were observed on both control treatments. *A. alternata* was reisolated from the inoculated leaves and confirmed by both morphological and molecular techniques, confirming Koch's postulates. To our knowledge, this is the first report of *A. alternata* causing leaf spot on *S. asper* in China. The pathogen could cause severe disease in *S. asper* and has the potential to be further studied as a fungal weed biocontrol.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Cho, M. S., et al. 2019. Genes (Basel) 10:881.
- Lawrence, D. P., et al. 2013. Mycologia 105:530.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Centre, Utrecht, the Netherlands.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Sonchus asper*, *Alternaria alternata*, leaf spot

[†]Indicates the corresponding author.

E-mail: H. Q. Cao; haiquncao@163.com