Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Alfalfa Anthracnose Caused by *Colletotrichum americanae-borealis* in Xinjiang, China

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Alfalfa (*Medicago sativa* L.) is widely planted as one of the most important leguminous forage crops, and it is also the top forage crop for animal feed in Xinjiang. In June 2018, alfalfa plants with typical anthracnose symptoms were observed in 75% of alfalfa fields in Hutubi County, Xinjiang, China. The disease usually occurred in alfalfa fields that had been planted for more than 2 years and was distributed in patches in the field. The incidence rate ranged from 7.5 to 53%, and the fatality rate ranged from 0 to 3%. Greater incidence was observed in fields with older stands. At the early stage of disease, pale brown prismatic or oval sunken lesions with dark brown to black edges were observed at the base of the stem of alfalfa plants. As the symptoms progressed, lesions on stems turned necrotic, and the center of the lesion became gray-white with black dots. In severe cases, the lesion expanded around the stem, causing the upper part of the stem to break off, or wilt and die. Twenty plant stem sections with typical symptoms were sampled and surface-sterilized with 75% ethanol for 30 s and 1% NaClO for 1 min, rinsed in sterilized distilled water, dried on sterilized filter paper for 45 s, placed on PDA, and incubated in the dark at 25°C for 7 days. A fungus was frequently isolated from the surface-sterilized segments, with colonies that were white and flat at first, and later the centers became pale brown with black microsclerotia (2.0 to 3.2 mm, $n = 30$) and white or brown acervuli (1.0 to 1.8 mm, $n = 30$). A large number of conidia and setae spread from ruptured microsclerotia under microscopy. Conidia ($n = 40$) were hyaline, smooth-walled, straight, aseptate, cylindrical to fusoid, both tips acute to round, 13.7 to 19.5 $\times$ 3.0 to 4.5 μ m. Setae ($n = 30$) were dark brown to black, smooth-walled, 3 to 6 septate, straight or slightly curved, 66.9 to 185.1 μ m long, tip round and base swollen, 3.9 to 5.2 μ m wide. Sometimes setae formed directly on hyphae or brown acervuli. Colony and

conidia morphology were similar to the description of *Colletotrichum americanae-borealis* (Damm et al. 2014; Lyu et al. 2020). DNA was extracted from fresh mycelia of three representative isolates (R11, R12, and R13) and the ITS, ACT, CHS-1, and HIS3 genes of three isolates were amplified and sequenced using the primers described previously by Damm et al. (2014). The sequences of three isolates were identical, and 12 aligned sequences from three representative isolates were deposited in GenBank (MT877442, MT877443, and MT877444 for ITS; MW854350, MW854351, and MW854352 for ACT; MW270930, MW270931, and MW270107 for CHS-1; MW854347, MW854348, and MW854349 for HIS3). Sequence analysis revealed that the ITS, ACT, CHS-1, and HIS3 sequences of three representative isolates shared 99% (355/356 bp for ITS) to 100% (550/550 bp for ITS, 261/261 bp for ACT, 221/221 bp for CHS-1) identities to each sequence of an American strain (CBS 136232) of *C. americanae-borealis* from alfalfa in GenBank (NR160760 for ITS, KM105434 for ACT, KM105294 for CHS-1, KM105364 for HIS3). Four phylogenetic trees were constructed by the MrBayes method (Damm et al. 2014), and the result showed that three representative isolates grouped with *C. americanae-borealis*. Combined with morphological observation and molecular biological identification, the pathogen was identified as *C. americanae-borealis*. Pathogenicity tests were executed twice on alfalfa seedlings in a greenhouse. Pots containing 10 40-day-old seedlings (Xinjiang daye) were sprayed with 100 ml of conidial suspension (10^6 conidia/ml) of R11. Control pots were sprayed with 100 ml of sterile distilled water. Two weeks after inoculation under greenhouse conditions ($25 \pm 2^\circ\text{C}$, 12-h photoperiod, 85% RH), brown spots and necrotic lesions developed on stems of inoculated alfalfa seedlings, similar to diseased plants in fields, and *C. americanae-borealis* was reisolated from symptomatic tissue. Control seedlings remained symptomless. Anthracnose caused by *C. americanae-borealis* was reported on alfalfa in the northern region of America and Iran (Alizadeh et al. 2015; Damm et al. 2014), as well as Gansu, Inner Mongolia, Yunnan, and Heilongjiang provinces of China (Xu 2019; Zhang et al. 2020). To our knowledge, this is the first report of *C. americanae-borealis* causing alfalfa anthracnose in Xinjiang, China. This finding can provide an important reference for understanding the distribution and control of this disease.

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