Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum cereale* Causing Anthracnose on *Avena nuda* in China

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Funding: Funding was provided by National Key Basic Research Program of China (2013CB127700, 2017YFD0300906, and 2018YFD0300502), Traditional Chinese Medicine Industry System (HBCT2018060204), Natural Science Foundation of Hebei Province (C2019204040), and Establishment of Rapid Detection Technology of Naked Oats Smut (ZD2019130). Plant Dis. 105:1216, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2109-PDN>. Accepted for publication 15 December 2020.

Naked oats (*Avena nuda* L.) are rich in protein, fat, vitamins, minerals, and so on and are one of the world's recognized cereal crops with the highest nutritional and healthcare value. In July 2019, leaf spot was detected on *A. nuda* in Zhangbei experimental station of Hebei Agricultural University. The incidence of disease was 10 to 20%. The symptoms were similar to anthracnose disease; the infected leaves had fusiform or nearly fusiform yellowish-brown spots with a yellow halo around the spots. Numerous acervuli with black setae diagnostic of fungi in the genus *Colletotrichum* were present on necrotic lesions. To identify the pathogen, 10 symptomatic leaves were collected, and only one disease spot was isolated from each leaf. Small square leaf pieces (3 to 5 mm) were excised from the junction of diseased and healthy tissues with a sterile scalpel and surface disinfested with 75% alcohol for 30 s followed by 0.1% corrosive sublimate for 1 min and rinsed three times in sterile water. Plant tissues were then transferred on potato dextrose agar and incubated at 25°C for 7 days. Two fungal isolates were obtained and purified by the single-spore isolation method. All fungi had the same morphology, and no other fungi were isolated. The aerial mycelium was gray black. The conidia were colorless and transparent, falcate, slightly curved, tapered toward the tips,

and produced in acervuli with brown setae. The length and width of 100 conidia were measured, and size ranged from 1.86 to 3.84 × 8.62 to 29.81 μm. These morphological characteristics were consistent with the description of *Colletotrichum cereale* (Crouch et al. 2006). To further assess the identity of the species, the genomic DNA of two fungal isolates (LYM19-4 and LYM19-10) was extracted by a CTAB protocol. The ribosomal DNA internal transcribed spacer (ITS) region as well as the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), and the beta-tubulin 2 (Tub2) partial genes were amplified and sequenced with primers ITS4/5, GDF/GDR, ACT-512F/ACT-783R, and T1/Bt2b, respectively (Carbone and Kohn 1999; Glass and Donaldson 1995; O'Donnell et al. 1997; Templeton et al. 1992). The sequences of the ITS-rDNA region (MW040121, MW040122), the GAPDH sequences (MW052554, MW052555), the ACT sequences (MW052556, MW052551), and the Tub2 sequences (MW052552, MW052553) of the two single-spore isolates were more than 99% identical to *C. cereale* isolate CGMCC3.15110 (JX625159, KC843517, KC843534, and JX625186). A maximum likelihood tree based on concatenated sequences of the four genes was constructed using MEGA7. The results showed the strains isolated from *A. nuda* were closely related to *C. cereale*, as supported by high bootstrap values. A pathogenicity test of the *C. cereale* isolates was performed on first unfolding leaves of *A. nuda*. Koch's postulates were carried out with isolates by spraying a conidial suspension of 10⁶ conidia/ml on leaves of healthy *A. nuda*. Four replicated pots were inoculated at a time, 10 leaves per pot, whereas sterile distilled water was used as the control. All treated plants were placed in a moist chamber (25°C, 16-h light and 8-h dark period). Anthracnose symptoms developed on the inoculated plants 7 days post inoculation, whereas all control plants remained healthy. Microscopic examination showed the surface of infected leaves had the same acervuli, setae, and conidia as the original isolate. The pathogenicity test was repeated three times. *C. cereale* was previously reported as the causal agent of anthracnose on feather reed grass in the United States (Crouch et al. 2009). To our knowledge, this is the first report of *C. cereale* as the causal agent of *A. nuda* anthracnose in China.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Crouch, J. A., et al. 2006. Phytopathology 96:46.
- Crouch, J. A., et al. 2009. Plant Dis. 93:203.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- O'Donnell, K., et al. 1997. Mol. Phylogenet. Evol. 7:103.
- Templeton, M. D., et al. 1992. Gene 122:225.

The author(s) declare no conflict of interest.

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Keywords: naked oats, fungi, *Colletotrichum cereale*

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