Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Ergot (*Claviceps purpurea*) on Drunken Horse Grass (*Achnatherum inebrians*) in China

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Drunken horse grass (*Achnatherum inebrians*) belongs to the family Poaceae; it is mainly distributed in the natural grasslands of northern and north-western in China. Ergot is a disease that can not only affect the growth of the grass but also cause livestock poisoning (Coufal-Majewski et al. 2016). In September 2018, ergot was observed in a large area (about 15 ha) in Xinghai county, Qinghai province, China (35.47° N, 99.53° E, altitude 3,559 m). Around 65% of the plants of *A. inebrians* were affected. Symptoms initially showed drop-like honeydew on the ears of drunken horse grass, and later brown to dark brown sclerotia were observed. These were straight to slightly curved and measured 6.7 to 13.5 × 1.5 to 2.1 mm, which was approximately one to four times the size of healthy seeds. Sixteen spikes with typical symptoms were collected from eight different fields. Sclerotia were disinfested by immersion in 75% ethanol for 30 s and 1% NaClO for 90 s, rinsed three times in sterilized water, plated on potato dextrose agar (PDA) medium, incubated at 24°C in the dark and isolates purified by culturing from single spores. Finally, 16 single-spore cultures with similar phenotypes were obtained from these sclerotia. Colonies produced on PDA for 15 days at 24°C were grayish white with fluffy aerial mycelium and were about 60 mm in diameter. Conidia were

hyaline, ovoid to cylindrical, and 5.42 to 7.69 × 2.85 to 3.75 µm (average 5.67 × 3.2 µm; *n* = 50). These morphological characteristics were consistent with the descriptions of *Claviceps* species in general (Píčová et al. 2018). To further identify the *Claviceps* spp., isolate NSZJ (= MHLZU-AI20201012) was selected as a representative for molecular characterization. Two nuclear protein-coding genes, *TUB2* and *MCM7*, were amplified by T2/T12 (O'Donnell and Cigelnik 1997) and CARCA-F/M456-5R (Rehner and Buckley 2005), respectively, and sequenced. Sequences were deposited in GenBank (accession nos. MW115640 for *TUB2* and MW115641 for *MCM7*). A BLAST analysis of these two segments showed >99% identity with those sequences of isolate W3 of *Claviceps purpurea* (Pažoutová et al. 2015). To confirm the pathogenicity on drunken horse grass, 20 healthy plants (2 years old) grown in an experimental field at the College of Pasture Agriculture Science and Technology, Yuzhong Campus of Lanzhou University in China (104.39° E, 35.89° N, altitude 1,653 m), were spray inoculated with conidial suspension (1 × 10⁶ conidia/ml) during the flowering period. Another 20 plants in the field were sprayed with sterilized distilled water as controls. All plants were individually covered with transparent polyethylene bags for 24 h to maintain high relative humidity. After 7 to 10 days, small yellowish-white drops of honeydew were observed in some florets. At 22 days postinoculation, all the inoculated panicles developed three to six sclerotia per head, ranging in size from 6.7 to 13.5 mm, whereas control plants remained healthy. The same pathogen was consistently reisolated from inoculated spikes and confirmed by morphological and molecular characterization as described above. *C. purpurea* was reported to be associated with ergot in *A. lemmonii* in Idaho and *A. robustum* in Montana (Alderman et al. 2004). To our knowledge, this is the first report of *C. purpurea* causing ergot in *A. inebrians* in China.

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