Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of Leaf Spot on *Avena sativa* Caused by *Canariomyces microsporus* in China

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Oats (*Avena sativa*) are an important fodder crop in the vast ranges of northern and northwestern China, given the growing demand from livestock (Yang et al. 2010). In July 2020, diseased leaf samples of cultivar Dingyan-2 were collected from fields near Gonghui Town, Zhangbei County, Zhangjiakou City (41.35° N, 114.55° E). These leaves showed oval to irregular yellowish-brown spots (0.5 to 6 mm in diameter) surrounded by a yellowish halo progressing to form narrowly striped spots fusing into lesions in severe cases. In a disease survey of six fields (about 1.5 ha in total), 35% of the plants were infected with a disease severity ranging from 0 to 20%. To isolate the pathogen, 12 symptomatic leaves (two leaves for each plant) were arbitrarily sampled from different locations across the fields, and small pieces (5 mm²) of diseased leaves were excised from the border between diseased and healthy tissue. Excised tissue pieces were surface sterilized by immersion in 75% ethanol for 30 s and then 1% NaClO solution for 1 min, rinsed in sterilized distilled water three times, and transferred to potato dextrose agar (PDA). Colonies on PDA were 41 to 46 mm in diameter in 10 days at 25°C with surface texture floccose, obverse pale mouse gray to black due to ascospores and aerial mycelium, and reverse pale olivaceous. Asci were ellipsoidal to ovoid, 12 to 18 × 11 to 15 µm (average = 15 × 12 µm; *n* = 30) in the spore-bearing part, containing eight irregularly arranged ascospores. Ascospores were one-celled, dark brown when mature, smooth, ellipsoidal, with attenuated ends, 7.5 to 8.4 × 4.3 to 5.5 µm (average = 8.1 × 5.0 µm; *n* = 50),

with an apical or slightly subapical germ pore. These morphological characteristics were consistent with previous descriptions of *Canariomyces microsporus* (syn. *Thielavia microspora*, Wang et al. 2019). For molecular identification, genomic DNA (isolate MNK-Y1) was extracted, and the internal transcribed spacer (ITS) region and β-tubulin (*tub2*) were amplified and sequenced by using the primers ITS1 and ITS4 (White et al. 1990) and Btub2Fd and Btub4Rd (Woudenberg et al. 2009). Sequences were deposited in GenBank under accessions MW080329 (ITS) and MW557539 (*tub2*). BLAST search revealed that the ITS and *tub2* sequences matched 99.4 and 100% (471 of 474 bp; 648 of 648 bp) with the sequences of the ex-type isolate CBS 276.74 of *C. microsporus* (accession nos. MH860852.1 and MK926899). Koch's postulates were proven to confirm the pathogenicity of isolate MNK-Y1. Eight-week-old healthy oat seedlings of cultivar Dingyan-2 were grown in the greenhouse, at 15 to 20°C under 30 to 40% relative humidity. Ten oat plants were spray inoculated with a spore suspension (5 × 10⁵ spores/ml; isolate MNK-Y1). Another 10 oat plants were sprayed with sterile water as controls. All plants were covered with a transparent glass cover and a black polyethylene bag to maintain relative humidity and dark for 2 days. After 15 days, all the inoculated plants had developed yellowish-brown spots similar to those observed in the field, whereas the control plants sprayed with sterile water remained healthy. The pathogen was reisolated from inoculated plants and identified as *C. microsporus* based on morphological characteristics and the molecular methods described above. This species has previously been isolated from saline and desert soils as well as from leaves of *Thymus* (Wang et al. 2019). To our knowledge, this is the first report of leaf spot of oat caused by *C. microsporus* in China.

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