Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Canker of *Betula alnoides* in China

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Betula alnoides Buch. Ham. ex D. Don is a valuable deciduous hardwood species mainly distributed in Southeast Asia and South China. It is a fast-growing, high-yielding timber species, and more than 150,000 ha has been planted in South China (Wang et al. 2016). Cankers were observed on *B. alnoides* saplings in Xijiang State-Owned Forest Farm, Guangdong Province (23°05'1"N, 111°53'56"E) in 2016. The initial symptoms were small, brown lesions and elongated oval-shaped cankers. Cankers caused branches to wither and defoliate, eventually leading to tree death. To isolate the causal agent, sections of branches were soaked in 1% NaOCl for 30 s, and fragments (approximately 2 mm) were taken from the margin of the internal diseased tissues, cultured on potato dextrose agar (PDA), and incubated in a chamber at 28°C under alternating light and dark cycles (14 h/10 h). Colonies of the fungus initially appeared whitish and then turned dark gray. Black pycnidia developed on the surface of PDA plates after 21 to 25 days of incubation. Conidia were one-celled, hyaline, aseptate, fusoid, and 17.19 to 23.74 µm (mean 18.93 µm) × 3.72 to 4.93 µm (mean 4.45 µm) ($n = 50$). These morphological characteristics are in

accordance with those described for the fungus *Botryosphaeria dothidea* (Crous et al. 2006; Zhai et al. 2014). Genomic DNA was isolated from the fungus, and the internal transcribed spacer (ITS) region of rDNA was amplified with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and sequenced. The nucleotide sequence has been assigned GenBank accession number MN826494. BLAST analysis of the 583-bp segment showed 99% similarity with *B. dothidea* isolates deposited in GenBank under accession numbers KC527822 and KY788307, which confirmed the above morphological identification. Pathogenicity tests were conducted on 2-year-old *B. alnoides* seedlings. Mycelial plugs (5 mm diameter) of *B. dothidea* from the margin of actively growing colonies (PDA) were applied to wedge-shaped bark wounds on branches ($\Phi = 15$ mm) with a scalpel. Inoculation wounds were covered with moist, sterile cotton and wrapped with Parafilm. Seedlings inoculated with plugs of PDA were treated as a control. Inoculated and control seedlings (five each) were grown in a greenhouse. The symptoms described above appeared on all inoculated seedlings 15 days after inoculation, whereas control seedlings remained healthy. *B. dothidea* was reisolated from symptomatic tissues, thus fulfilling Koch's postulates. To our knowledge, this is the first report of *B. dothidea* isolated from *B. alnoides* showing canker in China.

References:

- Crous, P. W., et al. 2006. Stud. Mycol. 55:235.
Gardes, M., and Bruns, T. D. 1993. Mol. Ecol. 2:113.
Wang, C. S., et al. 2016. For. Ecol. Manage. 375:200.
White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
Zhai, L. F., et al. 2014. Plant Dis. 98:716.

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