Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Stem Canker and Dieback of *Gleditsia sinensis* in China

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Gleditsia sinensis Lam is a kind of legume perennial woody plant, which is a traditional Chinese medicine with high economic and ecological value. Its planting area is about 0.1 million hectares. In July 2018, symptoms of stem blight were observed on *G. sinensis* in An shun (26.072311°N, 106.097433°E), Guizhou province (southwestern China). Symptoms included stem canker and dieback, twig blight, and extensive vascular discoloration, with incidence rate of 45 to 70%. Samples from plants with symptoms were washed with running tap water, surface sterilized with 2% sodium hypochlorite and then 75% ethanol, rinsed in sterile distilled water, plated on potato dextrose agar (PDA), and incubated at 28°C. Fungal isolates developed copious white aerial mycelium that became dark gray after 4 to 5 days and formed black pycnidia after 23 days. Single-hyphal-tip cultures of 27 putative isolates were stored in the culture collection (CMW) of the Urban Modern Agriculture Engineering Research Center at the Kunming University. Conidia forming on pycnidia were one-celled, hyaline, aseptate, and fusiform, with dimensions of 20.3 to 25.9 × 4.2 to 6.5 µm (average 22.5 × 5.5 µm) (60 conidia were measured). DNA sequence analysis of the ribosomal internal transcribed spacer regions ITS1-5.8S-ITS4, β-tubulin, and the translation elongation factor 1-α

(EF1-α) were performed. BLAST searches at GenBank showed the highest nucleotide sequence identity with *Botryosphaeria dothidea* reference sequence (ITS: >99.82%, KR708996; EF1-α: 99.62%, KP183214; β-tubulin: >99.67%, KU306116). Representative sequences of isolates from these regions were deposited in GenBank (ITS: MT449017; EF1-α: MT454342; β-tubulin: MT454343). Morphological and molecular results confirmed this species as *B. dothidea* (Aguirre et al. 2018). Pathogenicity tests were conducted by stem inoculation of 2-year-old *G. sinensis* seedlings. Mycelial plugs (2 to 3 mm in diameter) from actively growing colonies of *B. dothidea* (PDA) were applied to same-size bark wounds on the middle point of the stems. Inoculated wounds were wrapped with Parafilm. Control seedlings received sterile PDA plugs. Inoculated and control seedlings (nine each) were kept in the greenhouse (28°C, 80% humidity). After 10 days, all of the inoculated plants showed dark vascular stem tissue, and the controls remained healthy. After 30 days, all of the inoculated but none of the control *G. sinensis* seedlings showed leaf wilting and tissue discoloration. *B. dothidea* was reisolated from symptomatic tissues, thus fulfilling Koch's postulates. No symptoms were visible in the control seedlings, and no *B. dothidea* was reisolated from the control seedling tissues. *B. dothidea* is a member of Botryosphaeriaceae, commonly associated with cankers and dieback of woody plants. *B. dothidea* has been reported as a pathogen causing stem dieback and branch canker on *Malosma laurina* (Aguirre et al. 2018), *Helwingia chinensis* (Yu et al. 2012b), and blueberry (Choi 2011; Yu et al. 2012a). To our knowledge, this is the first report of *B. dothidea* on *G. sinensis* in China.

References:

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