

Note

pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 36, No. 4, pp. 326-327, December, 2020
<https://doi.org/10.7747/JFES.2020.36.4.326>

Molecular Confirmation of the Occurrence of *Botryosphaeria dothidea* Responsible for Branch Dieback and Canker on *Juglans sinensis* in South Korea

Dong-Hyeon Lee^{1,*}, Sung-Eun Cho², Ji Yeon Oh¹ and Sang-Hyun Lee¹

¹Division of Forest Diseases and Insect Pests, National Institute of Forest Science, Seoul 02455, Republic of Korea

²Forest Biodiversity Division, Korea National Arboretum, Pocheon 11186, Republic of Korea

Walnut is one of the important tree crops that is widely cultivated for nut production in South Korea. In 2018, five to six-year-old walnut trees, *Juglans sinensis*, that showed symptoms including branch dieback, necrotic lesions on branches, cankers on the shoots, and dark brown discoloration on the stems, were consistently observed in Andong, Buyeo and Yeongju regions (Supplementary Fig. 1). Ascostromata were found on the dead stems (Supplementary Fig. 1C). Asci were bitunicate, clavate or cylindrical, and contained eight hyaline and aseptate ascospores. These structures were identical to those described by Phillips et al. (2013). Among the isolates that were consistently retained, three representative single spore isolates obtained from each region were selected and deposited to the culture collection of the National Institute of Forest Science, South Korea (Accession nos. CDH2019-1-3). Genomic DNA was extracted from the mycelium of these isolates following the technique described by and rDNA ITS, beta-tubulin subunit 2 (β -tubulin) and translation elongation factor-1 alpha (*TEF-1 α*) gene regions were sequenced using the respective primer pairs ITS1/ITS4, Bt2a/Bt2b and EF1-728F/EF1-986R. The species identification was performed by BLAST searches in GenBank selecting sequences of key isolates from pub-

lished studies, including the ex-type isolate. The sequences of ITS (GenBank Accession Nos. MK391933 to MK391935), β -tubulin (MK783289 to MK783291) and *TEF-1 α* (MK783292 to MK783294) revealed 100% similarity with those of *Botryosphaeria dothidea* (CBS 115476 for ITS, CBS 110302 for β -tubulin and *TEF-1 α*) (Phillips et al. 2013), confirming its identity. Three healthy young branches of seven trees selected in the field were subjected to the inoculation tests following the technique described by Oh et al. (2020). After six weeks, symptoms from all inoculated trees were developed, identical to those described above, while the control did not show any symptoms. The fungus was successfully re-isolated from the lesions, fulfilling Koch's postulates. *Botryosphaeria dothidea* has previously been reported in South Korea, which was responsible for canker and dieback on *J. sinensis* (Lee et al. 1992). However, the identification of the pathogen was made based only on the cultural and morphological characteristics. There have been substantial changes in the taxonomic circumscription of *B. dothidea* and many of the specimens that had been reported as *B. dothidea* revealed that it belongs to other species since the advent of DNA based identification (Marsberg et al. 2017). In this regard, this study first confirmed the occurrence of *B. dothidea* responsible for branch dieback

Received: October 8, 2020. Revised: November 10, 2020. Accepted: November 13, 2020.

Corresponding author: Dong-Hyeon Lee

Division of Forest Diseases and Insect Pests, National Institute of Forest Science, Seoul 02455, Republic of Korea
Tel: +82-2-961-2673, Fax: +82-2-961-2679, E-mail: leedh2009@korea.kr

and canker on *J. sinensis* in South Korea based on the morphological and molecular identification.

Acknowledgements

The authors acknowledge the financial support from the National Institute of Forest Science, 'Development of management tools for insect pests and fungal pathogens occurring on forest tree crops, no. FP0802-2017-02'.

Ethical Statements

All the authors have sufficiently contributed to the work, have agreed to this submission and are responsible for its contents. This manuscript including the data that are supporting the aim and the conclusion of this research is new and is not being considered elsewhere. No data have been fabricated or manipulated for being published to the journal.

Conflict of interest

The authors have no conflicts of interest to declare.

References

- Lee MO, Lee YH, Cho WD, Lee KJ. 1992. Identification of Three Fungi Associated with Stem and Twig Diseases of *Juglans sinensis* in Korea and Characterization of Factors Affecting Their Growth. *J Korean For Soc* 81: 191-199.
- Marsberg A, Kemler M, Jami F, Nagel JH, Postma-Smidt A, Naidoo S, Wingfield MJ, Crous PW, Spatafora JW, Hesse CN, Robbertse B, Slippers B. 2017. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Mol Plant Pathol* 18: 477-488.
- Oh JY, Lee DH, Chung RJ, Kim CW, Lee SH. 2020. First Report of Root and Collar Rot Caused by *Neocosmospora solani* on *Ligusticum chuanxiong* in South Korea. *J For Environ Sci* 36: 173-174.
- Phillips AJ, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW. 2013. The Botryosphaeriaceae: genera and species known from culture. *Stud Mycol* 76: 51-167.