Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* as a Causal Agent to Stem Rot Disease on Plumcot Trees in Korea

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Botryosphaeria dothidea is a fungal pathogen commonly associated with stem canker, dieback, and rot disease in a variety of woody plants worldwide (Dong and Guo 2020). In Korea, *B. dothidea* was reported to cause a disease in several crops such as apple and blueberry (Choi 2011; Kim 1995). In early 2020, a symptom resembling stem rot disease occurred at a plumcot cultivation area around Wanju Province (35.827870°N, 127.030380°E), Korea. At the early stage of infection, a small blister appeared on the plumcot branch and stem. As the blister extended, a light brown canker appeared on the infected area and in some cases a sticky sap oozed from the branch bark crack. If not managed or treated properly, all leaves beyond the infection site turned brown, wilted, and the whole plumcot tree eventually died. A survey in the affected area showed that approximately 5% of the plumcot trees were infected, which caused up to a 10% reduction in total production. To identify the causal agent, symptomatic tissues were excised and surface sterilized with 70% ethanol for 30 s followed by 1% NaClO for 30 s before rinsing with sterile water. The samples were then dried with filter paper and air-dried before being placed on potato dextrose agar (PDA). The PDA plates were then incubated at 25°C for 5 days with a 12 h light/dark cycle. Four fungal isolates were selected for further analyses. Conidia were hyaline, ovoid, fusiform, and unicellular with an average size of 18 to 20 µm × 4.5 to 5.5 µm ($n = 50$). These morphological characters have a strong resemblance to *B. dothidea* (Slippers et al. 2004). For molecular identification, internal transcribed spacer (*ITS*), beta-tubulin (*β-tubulin*), and elongation factor 1 alpha (*EF-1α*) were amplified and sequenced using universal primer pairs ITS1/ITS4 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), and EF1/EF2 (O'Donnell et al. 1998), respectively. Alignment analysis

showed that *ITS* (LC602817), *β-tubulin* (LC602820), and *EF-1α* (LC602821) sequences were 99 to 100% identical to the orthologous genes identified in *B. dothidea* infecting soybean in China (MW130133 [identity 537/536 bp], MW147482 [identity 394/394 bp], and MW147481 [identity 250/250 bp], respectively) (Chen et al. 2021). However, phylogenetic analysis of concatenated *ITS*, *β-tubulin*, and *EF-1α* sequences established the identity of these isolate as *B. dothidea*. Due to its 100% identity at the molecular level, isolate NIHHS 20-262 was selected as a representative for further analysis. For the pathogenicity test, fungal mycelium (via PDA plug) was used as a source of inoculum for both intact and detached plumcot stem trials. For the intact trial, mycelium was inoculated on the wounded spots of 10 plumcot stems that grew at the NIHHS trial farm. Ten days post inoculation, disease symptoms were observed at the inoculated sites. For the detached trial, mycelium was inoculated on the wounded spots of 10 detached plumcot stems. The inoculated stems were kept in a closed container to maintain 90% humidity before incubation at 25°C in the dark. Interestingly, on the detached stems, disease symptoms appeared 7 days earlier than with intact stems. A sterile PDA plug served as a negative control and the result shows no symptoms on either intact or detached stems. Pathogenicity tests on intact stems were performed on three different plumcot trees, whereas three biological replicates were used for detached stems. Isolation and reidentification of two colonies from the infected sites (intact and detached stems) were attempted and the results obtained were identical to the original isolate, thus fulfilling Koch's postulates. Local farmers described this disease as "certain death disease" in plumcot. Therefore, accurate identification of *B. dothidea* as the causal agent is critical for effective disease management to minimize qualitative and quantitative losses in the plumcot industry. Although has been reported to cause dieback disease in blueberry in Korea (Choi 2011), to our knowledge, this is the first study to report *B. dothidea* causing stem rot diseases on plumcot trees in Korea.

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