Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Branch Dieback on Castor Bean in Zhanjiang, China

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Funding: This research was supported by Postgraduate Education Innovation Program of Guangdong Ocean University (202034). Plant Dis. 105:2713, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1723-PDN>. Accepted for publication 22 April 2021.

Castor bean (*Ricinus communis* L.) is an oil crop of significant economic importance in industry and medicine. In August 2019, a branch dieback disease was observed on castor bean in a field in Zhanjiang (21.17°N, 110.18°E), China. The incidence rate was 35% ($n = 600$ investigated plants). Symptoms were discoloration of leaves, branch dieback, and discoloration of internal stem tissues. The disease had spread to whole branches and caused the plant to die. Seven diseased branches were collected from seven plants. Margins between healthy and diseased tissues were cut into 2 mm × 2 mm pieces. The surfaces were disinfested with 75% ethanol for 30 s and 2% sodium hypochlorite for 60 s. Then, the samples were rinsed three times in sterile water, placed on potato dextrose agar (PDA), and incubated at 28°C. Pure cultures were obtained by transferring the hyphal tips to new PDA plates. Eighteen isolates were obtained (the isolate rate of 75%), which were identified as the same fungus on the basis of morphological characteristics and molecular analysis of the internal transcribed spacer (ITS). A single representative isolate (RiB-1) was used for further study. The colony of RiB-1 was 5 cm in diameter on the fifth day on the PDA culture. The colony was greenish gray with an irregularly distributed and fluffy aerial mycelium, which turned black after 10 days. The mature conidia were 21.3 to 26.5 μm × 12.2 to 15.7 μm in size ($n = 100$) and had two ovoid, dark brown cells with longitudinal striations. The morphological characteristics of the colonies were consistent with the description of *Lasiodiplodia* sp. (Alves et al. 2008). Three regions of the ITS, translation elongation factor (*EF1-α*), and *β-tubulin* genes were amplified and sequenced

with the primer pairs ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Alves et al. 2008), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The resulting sequences were deposited in GenBank under accession numbers MN759432 (ITS), MN719125 (*EF1-α*), and MN719128 (*β-tubulin*). BLASTn analysis demonstrated that these sequences were 100% identical to the corresponding ITS (MK530052), *EF1-α* (MK423878), and *β-tubulin* (MN172230) sequences of *L. theobromae*. Based on the morphological and molecular data, RiB-1 was determined as *L. theobromae*. A pathogenicity test was performed in a greenhouse with 80% relative humidity at 25 to 30°C. Ten healthy plants of Zi Bi No. 5 castor bean (1 month old) were grown in pots with one plant in each pot. Five pots were wound-inoculated with 5-mm-diameter mycelial plugs obtained from 7-day cultures. Five additional pots treated with PDA plugs served as the controls. Inoculated stems were moisturized with sterile cotton for 5 days. The test was conducted three times. Disease symptoms, similar to those in the field, were observed on the inoculated plants 2 weeks after inoculation, and *L. theobromae* was 100% reisolated from the inoculated plants. The control plants remained symptomless and reisolations were unsuccessful. These results were consistent with Koch's postulates. *L. theobromae* (Lima et al. 1997) and *L. hormozganensis* (Custódio et al. 2018) have been reported to cause stem rot on castor bean in Brazil, but whether *L. theobromae* caused the branch dieback on castor bean in China has not been reported yet. Thus, this study is the first report of *L. theobromae* causing branch dieback on castor bean in Zhanjiang, China. This study provides an important reference for the control of the disease.

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The author(s) declare no conflict of interest.

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Keywords: *Ricinus communis*, branch blight, phylogenetic analysis

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