Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Corynespora cassiicola* on *Acanthus ilicifolius* in China

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Acanthus ilicifolius, also referred to as holly mangrove, usually grows in the coastal areas in southern China. It is not only a pioneer species of mangroves but also an important medicinal mangrove plant. In June 2019, leaf spots on *A. ilicifolius* with disease incidence of ~30 to 40% were observed in about 150 plants in a field located in Zhanjiang City, Guangdong Province, China. The disease mainly occurred on the leaf margins or tips. Initial symptoms of the disease were dark brown spots (5 to 9 mm) surrounded by a yellow halo, that expanded irregularly or semicircularly, until finally the leaves turned chlorotic and defoliated. Six samples of symptomatic leaves were excised, surface sterilized with 75% ethanol for 30 s followed by 0.1% HgCl₂ for 30 s, rinsed three times with sterile water, cut into small pieces (5 × 5 mm), and placed on potato dextrose agar (PDA) at 25°C and darkness. Two isolates with different morphological characteristics were obtained after 3 days. For pathogenicity tests of the isolates, wounded and nonwounded leaves were inoculated. Fresh wounds were made with a sterile needle. Mycelial plugs from 8-day-old cultures of two isolates, ALY1 and ALY2, on PDA were inoculated on the adaxial surface of wounded and nonwounded healthy young leaves of the *A. ilicifolius* seedlings (2 years old, four leaves per plant, two replicate plants per isolate), covered with wet cotton, and kept in moist chambers at 25 ± 1°C and 80% relative humidity. Leaves inoculated with sterile PDA plugs were used as the control. After 3 to 5 days, the wounded and nonwounded leaves inoculated with ALY1 showed symptoms of leaf spots similar to those observed in the field, whereas leaves inoculated with ALY2 and the control leaves remained symptomless. The pathogenicity tests were repeated three times under the same conditions, and similar results were observed. ALY1 was confirmed as the pathogen causing the leaf spot. On PDA, ALY1 produced a gray to grayish brown colony. The conidia were obclavate to cylindrical, straight to slightly curved, pale brown, with six to 18 pseudosepta, 62.5 to 225.0 × 3.8 to 14.8 μm (*n* = 17).

According to the morphological characteristics, ALY1 was preliminarily identified as *Corynespora cassiicola* (Zhang et al. 2018). For molecular identification, the internal transcribed spacer (ITS), the actin gene (ACT), the β-tubulin gene (TUB2), and translation elongation factor 1-α gene (TEF1-α) of the three single-spore isolates from ALY1 (ALY1-1 to ALY1-3) were amplified and sequenced with primers ITS4/ITS5 (White et al. 1990), ACT512F/ACT783R (Carbone and Kohn 1999), T1/T2 (Glass and Donaldson 1995), and EF1-728F/EF1-986R (O'Donnell et al. 1998), respectively. The ITS region of ALY1 (-1, -2, -3) (GenBank accession no. MN860006, -07, -08) was 99 to 100% identical (579/585, 586/586, and 581/584 base pairs) to a corresponding ITS (MH255527) of *C. cassiicola*. Similarly, the ACT (MN887504, -05, -06), TUB2 (MN887507, -08, -09), and TEF1-α (MN887501, -02, -03) were 100% (342/342, 342/342, and 342/342 base pairs), 100% (664/664, 662/662, and 664/664 base pairs), and 100% (290/290, 290/290, and 290/290 base pairs) to corresponding genes FJ853033, MH763700, and MK589886 sequences of *C. cassiicola* in GenBank, respectively. A phylogenetic tree generated with MEGA7 by neighbor-joining analysis revealed that the isolate's ITS, ACT, TUB2, and TEF1-α sequences were grouped in the same clade as *C. cassiicola*. Based on both the morphological characteristics and sequences analyses, the pathogen causing leaf spot of *A. ilicifolius* was identified as *C. cassiicola*. This species had been reported on *A. ilicifolius* in Guam (U.S.A.) and Hong Kong (China) (Dixon et al. 2009; Lu et al. 2000; Sumabat et al. 2018; Zhuang 2001). To the best of our knowledge, this is the first report of *C. cassiicola* causing leaf spot of *A. ilicifolius* in China. This disease may be a potential threat to *A. ilicifolius* along the coastal regions of southern China.

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