Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe longicolla* Causing Leaf Spot on *Kalanchoe pinnata* in China

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Kalanchoe pinnata (Lam.) Pers. (syn.: *Bryophyllum pinnatum* [Lam.] Oken) is an important medicinal agent in southern China. The succulent leaves of this plant are used in the treatment of cholera, bruises, urinary diseases, and whitlow. In October 2019, leaf spots were detected on *K. pinnata* plants in Chengmai County, Hainan Province, China. Lesions with brown to black margins were irregularly shaped and associated with leaf margins. Spots coalesced to form larger lesions, with black pycnidia present in more mature lesions. Symptomatic *K. pinnata* were found with 10 to 20% incidence during the humid winters of Hainan Province. Leaf tissues of 10 symptomatic plants were collected and surface sterilized in 70% EtOH for 30 s followed by 0.1% HgCl₂ for 30 s, rinsed three times with sterile distilled water for 30 s, placed on potato dextrose agar (PDA) amended with 30 mg/liter of kanamycin sulfate, and incubated at 25°C in the dark for 3 to 5 days. Four fungal isolates were obtained using a single-spore isolation method. The colonies were floccose, dense, and white with forming on older

colonies grown on PDA. Alpha conidia exuded from ostiole, rostrate, long-beaked pycnidia in creamy-to-yellowish drops. Alpha conidia were hyaline, ellipsoidal, separated, and averaged 6.3 µm (SD ± 1.13 µm) long × 1.9 µm (SD ± 0.33 µm) wide (*n* = 50). Beta conidia were not seen. The morphological characteristics matched the previous description of *Diaporthe longicolla* (syn. *Phomopsis longicolla*) (Hobbs et al. 1985). Mycelial genomic DNA of the representative isolate LDSG3-2 was extracted as a template. The internal transcribed spacer (ITS), translation elongation factor 1α gene (TEF), and β-tubulin (TUB2) regions were amplified. These loci were amplified using primer pairs ITS4/ITS5 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. A BLAST search of GenBank showed ITS (MN960195), TEF (MN974483), and TUB2 (MN974482) sequences of the isolate were 99, 100, and 99% homologous with *D. longicolla* strains DL11 (MF125048, 557/563 bp), D55 (MN584792, 347/347 bp), and DPC-HOH-32 (MK161506, 502/504 bp). Maximum likelihood trees based on concatenated nucleotide sequences of the three genes were constructed using MEGA 7.0, and bootstrap values indicated the isolate was *D. longicolla*. Pathogenicity testing was performed using isolate LDSG3-2 by depositing 5-µl droplets of a conidial suspension (1 × 10⁶ conidia/ml) into five artificially wounded leaves (using a sterile needle) of 10 healthy 3-month-old *K. pinnata* plants. An equal number of artificially wounded control leaves were inoculated with sterile water to serve as a negative control. The test was conducted three times. Plants were kept at 25°C in 80% relative humidity and observed for symptoms. Two weeks after inoculation, no symptoms were observed on control plants, and all inoculated plants showed symptoms similar to those observed in the field. The fungus was reisolated from the infected tissues and showed the same cultural and morphological characteristics of the strain inoculated and could not be isolated from the controls, fulfilling Koch's postulates. To our knowledge, this is the first report of leaf spot on *K. pinnata* caused by *D. longicolla* in China. This disease is of concern because *Phomopsis* diseases are common in *K. pinnata* fields and can cause significant reduction in yield.

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