Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum siamense* Causing Anthracnose on *Erythrina crista-galli* in China

M. Li,¹ Z. Y. Gao,¹ X. Y. Hong,¹ S. G. Zhang,² C. Zhao,² and M. J. Hu^{1,†}

¹ Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences/Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture and Rural Affairs, Haikou, 571101, China

² School of Life and Pharmaceutical Sciences, Hainan University, Haikou, 570228, China

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Erythrina crista-galli L. (Fabaceae) is a popular ornamental plant in tropical and subtropical regions of South Asia. In October 2019, anthracnose-like lesions were observed on the leaves of *E. crista-galli* planted in Haikou, China. Five to 30% of leaves were infected. At first, the circular spots of 1 to 2 mm in diameter were reddish-brown on the leaves, and they then enlarged to circular, subcircular, or irregular spots with a reddish-brown center and surrounded by a diffuse yellow margin. Neighboring spots sometimes coalesced. Under continuously wet or humid conditions, the lesions expanded quickly and became gray, subcircular, or irregular spots covered by grayish-white mycelium and orange-pink conidial masses. Diseased leaves eventually fell off the trees. To identify the pathogen, diseased leaves were sampled from four gardens. Leaf tissues (5 × 5 mm) were cut from the margins of typical symptomatic lesions, surface sterilized in 1% sodium hypochlorite for 1 min, plated on potato dextrose agar (PDA) medium, and incubated at 28.0 ± 0.5°C in the dark. Similar fungal colonies were obtained from all plated tissues after 3 days. The single-conidium colonies of all isolates were white to pale gray and cottony with visible orange conidial masses. Conidia were one-celled, aseptate, hyaline, straight, cylindrical to fusiform with obtuse ends, and were 14.2 to 18.6 (16.4) × 3.8 to 5.4 (4.7) µm (*n* = 100). After germination, conidia formed single, brown, oval, or slightly irregular appressoria ranging from 8.0 to 11.8 (9.6) µm and from 4.8 to 6.0 (5.4) µm. Sexual stage was absent. These characteristics of conidia and appressoria matched

Colletotrichum siamense belonging to the *C. gloeosporioides* complex (Hu et al. 2015; Prihastuti et al. 2009; Weir et al. 2012; Yang et al. 2009). To accurately identify the species, DNA was extracted from four purified isolates (JG-1, JG-3-1, SWS-1-3, and SWS-2-1) (Fu et al. 2019). The internal transcribed spacer of rDNA region (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), calmodulin (CAL), actin (ACT), and chitin synthase (CHS) genes were amplified and sequenced. The nucleotide sequences were all deposited in GenBank (ITS, MT229427 to MT229430; GAPDH, MT250821 to MT250824; CAL, MT258893 to MT258896; ACT, MT258897 to MT258900; and CHS, MT258901 to MT258904). Multilocus phylogenetic analyses (ITS, GAPDH, CAL, ACT, and CHS) (Weir et al. 2012) showed that the four isolates were clustered with *C. siamense*, which was in accordance with BLAST results. Pathogenicity tests of the four isolates were repeated three times on detached leaves (Ji et al. 2019). The conidial suspension (1 × 10⁶ conidia/ml) was prepared using the conidia from 10-day-old cultures grown on PDA. Two 20-µl drops of conidial suspension were inoculated on nonwounded young healthy leaves, and each isolate was inoculated on 10 leaves. Two 20-µl drops of sterile water were inoculated on nonwounded young healthy leaves as a control. The samples were maintained in containers at a relative humidity of 90 ± 5% inside and 28°C with a 12-h photoperiod. Gray, subcircular spots similar to the field disease symptoms were observed on all the inoculated leaves after 7 days, whereas no visible symptoms appeared on the noninoculated leaves. The pathogen was reisolated from inoculated leaves, thus fulfilling Koch's postulates. *C. gloeosporioides* has been previously reported as a pathogen causing leaf spot on *Erythrina* (*E. indica* var. *picta*, *E. variegata* var. *orientalis*) in Guam in 1983 and Brazil in 2012. (Oliveira et al. 2012; Russo et al. 1983). To our knowledge, this is the first report of *C. siamense* causing leaf spot of *E. crista-galli* in China.

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[†]Indicates the corresponding author.

E-mail: M. J. Hu; humeijiao320@163.com