Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum* Leaf Spot of Macadamia Caused by *Colletotrichum siamense* in China

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Macadamia (*Macadamia integrifolia* Maiden & Betche), growing in tropical and subtropical regions worldwide, is a high-value tree nut (Akisanmi and Drenth 2017). In May 2018, the leaves of macadamia trees showed symptoms of anthracnose, with an incidence 10 to 15%, in Chongzuo (22°32' N; 107°38' E) (Guangxi province) in China. The disease began to develop from the tip of the leaf or the margin of the leaf. Initially, the disease symptoms on leaves were water-soaked, black-gray spots with light yellow around, which enlarged and coalesced; the leaves appeared irregular, with tawny or black lesions, ending in necrosis and defoliation. Abundant small black spots (acervuli) developed on the leaf surface, colonizing mainly the lesion zones. Under high-humidity conditions, lots of mucilaginous masses of pink conidia were observed on the leaf surface. Symptomatic leaves were randomly sampled from different parts of the field and washed with tap water. Tissues from the margins of the lesions were surface sterilized with 75% ethanol for 20 s followed by 2% sodium hypochlorite solution for 2 min, rinsed in sterile distilled water, plated on potato dextrose agar (PDA), and incubated at 28°C in constant light. After incubation for 5 days, the colonies of all isolates were white. After 10 days, a few aerial mycelia became greyish-green on the reverse side of plates. Fourteen days after incubation, many mucilaginous masses of pink conidia developed, and monoconidial isolates were obtained by single sporing. The nonseptate conidia were hyaline, cylindrical, obtuse at both ends, 14.4 to

21.4 µm (average 16.8 µm) × 5.4 to 7.0 µm (average 6.3 µm) ($n = 100$). These morphological characteristics were similar to the *Colletotrichum gloeosporioides* species complex (Weir et al. 2012). To confirm the pathogen's identity, DNA sequencing of the internal transcribed spacer (ITS), β -tubulin (TUB), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and histone H3 (HIS3) genes were performed. The sequences have been deposited in GenBank under accession numbers MT219898, MT227807, MT227808, MT227810, and MT227811, respectively. A BLAST of the ITS, TUB, ACT, GAPDH, and HIS3 sequences revealed 99 to 100% identity with those of *Colletotrichum siamense* (MG830368, MG830342, MH290847, MK863031, and MH370542). A phylogenetic tree generated using the maximum likelihood method in MEGA7.0 (Kumar et al. 2016) revealed that our isolate MICS0319 falls within the *C. siamense* clade. For the pathogenicity test, 10 healthy leaves of macadamia were slightly punctured by a sterile pin and inoculated with 5-mm-diameter mycelial plugs. The same number of leaves treated with PDA plugs served as controls. All plants were incubated at 28°C and 100% relative humidity. After 4 days, the leaves inoculated with mycelial discs showed water-soaked, tawny, necrotic lesions, whereas controls remained asymptomatic. The fungus was successfully reisolated from the symptomatic leaves, and the identity was confirmed by morphology and phylogeny as *C. siamense* on the basis of the methods described above, thus confirming Koch's postulates. *C. siamense* has been reported on macadamia, chili, papaya, and avocado (De Silva et al. 2017; Prasannath et al. 2020). To the best of our knowledge, this is the first report of *C. siamense* attacking leaf of macadamia in China. This research provides a basis for further research on the control of field disease.

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