Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Epicoccum nigrum* Causing Brown Leaf Spot in Tea in Guizhou Province, China

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Brown leaf spots were observed on tea (*Camellia sinensis* [L. O.] Kuntze) in Sinan County (27.74°N, 108.35°E) and Kaiyang County (27.96°N, 107.34°E), Guizhou Province, China, from 2018 to 2020. For leaf spots with typical symptoms, the disease incidence was estimated to range between 56 and 61%, respectively. The disease severity was estimated to range from 39 to 43 across 12 tea plantations, respectively. The disease initially occurred at the margins of leaf tips, and the lesions expanded gradually, being dark brown and irregularly shaped and becoming necrotic. To identify the causal organism, two leaves from each of 15 tea twigs, one or two per plantation, were detached from 8- or 10-year-old tea plants on each of 12 plantations. Samples taken from the lesion margins were sterilized with 75% ethanol followed by 0.5% NaOCl, placed on potato dextrose agar (PDA), and then incubated at 25°C in darkness for 5 days (Wang et al. 2020). For each sample, hyphal tips from the margin of a growing colony were successively transferred to fresh PDA, and pure cultures were obtained. Three representative strains were grown on PDA, malt extract agar (MEA), and oatmeal agar (OA) plates. The colonies had smooth margins and abundant mycelia on all three media, with the colony colors being from gray to light purple on PDA, white on MEA, and purplish-red on OA at 5 days postinoculation. At 20 days postinoculation on MEA, stromata began to gradually form, which were droplet-like, 100 to 2,000 µm in diameter, and semi-immersed on the medium's surface. Black sporodochia were produced on the surfaces of stromata. Conidiophores were aggregated in sporodochia, densely compacted, and dark brown. Conidia were globose or pyriform, dark, multicellular, and measured $22.95 \pm 3.59 \times$

$19.82 \pm 3.13 \mu\text{m}$ ($n = 50$) in diameter. The morphological characteristics of the mycelia and reproductive structures of the strains were identical to those of *Epicoccum nigrum* (Sheikhloo et al. 2011). The internal transcribed spacer (ITS) region of rDNA, and the partial 28S large subunit rDNA (*LSU*), RNA polymerase II second largest subunit (*RPB2*), and beta-tubulin (*TUB*) genes of these strains were amplified using the primers V9G/ITS4 (De Hoog and Gerrits van den Ende 1998; White et al. 1990), LR0R/LR5 (Rehner and Samuels 1994), RPB2-5F2/IRPB2-7cR (Sung et al. 2007), and TUB2Fd/TUB4Rd (Woudenberg et al. 2009), respectively, and deposited in GenBank (accession nos. MW646378, MW291537, MW602293, and MW602295 for ITS, *LSU*, *RBP2*, and *TUB*, respectively). A maximum parsimony phylogenetic analysis indicated that the representative strains clustered with *E. nigrum* CBS 173.73 (Chen et al. 2017). Pathogenicity tests were performed on 5-year-old potted tea and on 10-year-old *C. sinensis* cv. Fuding-dabaicha in the field. Mycelial plugs (6-mm diameter) and a conidial suspension (10^6 conidia/ml) were applied on punctured leaves using a sterile needle and non-punctured leaves. Inoculation with only a PDA plug or sterile water served as controls. Brown spots appeared on the wounded sites of tea leaves at 2 days postinoculation. No symptoms were observed on the nonwounded leaves or wounded leaves inoculated with PDA plugs lacking mycelia. The reisolated pathogen from diseased plants was identical to the purified strain ACCC39731 used for inoculation, with reisolation frequency being 85.0%. To our knowledge, this is the first report of *E. nigrum* causing leaf spot on tea plants in China, and our findings will be useful for its management and further research.

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The author(s) declare no conflict of interest.

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