Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Paramyrothecium breviseta* Causing Leaf Spot Disease of *Coffea canephora* in China

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Two coffee species are widely cultivated, namely *Coffea arabica* and *C. canephora*. An outbreak of a leaf spot disease on *C. canephora* seedlings was noticed in a nursery in Ruili County, Yunnan Province, China, during 2018 to 2019. The incidence of the disease was 15 to 20%. Ten symptomatic leaves from five distinct plants were collected for pathogen isolation. Leaf pieces (4 × 6 mm) were cut from the margin of necrotic lesions, surface-sterilized for 30 s in 75% ethanol, followed by 0.1% arsenic mercury solution for 15 s, washed 3 to 4 times with sterile distilled water, and transferred onto potato dextrose agar (PDA) medium in Petri dishes. Four morphologically similar isolates were obtained. Colonies of isolates were round, with a neat edge and a white floccose mycelium; they formed dark green to black concentric rings of sporodochia bearing viscous spore masses after 5 to 7 days. Conidia were acetates, hyaline and cylindrical with both ends rounded, 4.8 to 6.4 μm long × 1.6 to 2.6 μm wide. Pathogenicity tests were conducted on leaves of both *C. canephora* variety No. 2 and *C. arabica* variety Catimor C1FC7963 plants using a spore suspension (1 × 10⁶/ml). Three plants per each variety

were inoculated. An equal number of healthy plants of each variety inoculated with water served as a control. After inoculation, plants were kept in an incubator at 25°C with saturated humidity. Ten days after inoculation, all inoculated plants showed leaf symptoms similar to those observed in natural conditions, whereas the controls remained healthy. Koch's postulates were fulfilled by reisolating the fungus from symptomatic leaves. Two single spore isolates cultured on PDA were selected for DNA extraction. Total DNA was extracted by a kit (E.Z.N.A. Fungal DNA Kit, Omega Bio-tek, Inc., Norcross, GA) following the manufacturer's instructions. The ribosomal internal transcribed spacer (ITS) was PCR amplified using primers ITS1 and ITS4 (White et al. 1990), β-tubulin gene by Bt2a and Bt2b (Glass and Donaldson 1995), the RNA polymerase II second largest subunit (*rpb2*) by RPB2-5F2 and RPB2-7cR (O'Donnell et al. 2007), and the calmodulin (*cmla*) gene by CAL-228F and CAL2Rd (Groenewald et al. 2013). The sequences of ITS (MT853067–MT853068), β-tubulin (MT897899–MT897900), *rpb2* (MW256264–MW286265), and *cmla* (MT897897–MT897898) were deposited in GenBank. BLAST analysis revealed that the sequences of the isolates shared 99.31 to 99.65% similarities to the ITS sequence of *Paramyrothecium breviseta* (accession no. NR_155670.1), 99.43% similarities to the β-tubulin sequence of *P. breviseta* (accession no. KU846406.1), 98.98% similarities to the *rpb2* sequence of *P. breviseta* (accession no. KU846351.1), and 98.54 to 98.71% similarities to the *cmla* sequence of *P. breviseta* (accession no. KU846262.1). In the phylogenetic tree based on ITS, β-tubulin, *rpb2*, and *cmla* gene sequences, the two isolates clustered with *P. breviseta* CBS 544.75, confirming that *P. breviseta* is the causal agent of leaf spot of *C. canephora*. To our knowledge, this is the first report of a leaf spot disease caused by *P. breviseta* on *C. canephora* in China and also indicates that *C. arabica* may be a potential host of this pathogen.

References:

- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Groenewald, J. Z., et al. 2013. Stud. Mycol. 75:115.
- O'Donnell, K., et al. 2007. J. Clin. Microbiol. 45:2235.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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