Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neopestalotiopsis protearum* Causing Seed Rot on *Camellia oleifera* in China

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Funding: Funding was provided by the introduction of talent research start-up fund project of CSUF (2019YJ025). Plant Dis. 105:4152, 2021; published online as <https://doi.org/10.1094/PDIS-12-20-2717-PDN>. Accepted for publication 20 June 2021.

Camellia oleifera, an evergreen small tree or shrub with high medicinal and ecological values, is mainly distributed in subtropical regions of China. Camellia oil obtained from *C. oleifera* seeds is rich in unsaturated fatty acids and unique flavors, and it has become a rising high-quality edible vegetable oil in the south of China (Zhuang 2008). The tea-oil tree *C. oleifera* plays important economic and ecological roles in Hunan Province. During collecting trips, seeds of *C. oleifera* with disease symptoms were observed in almost all oil-tea forests. In the lab, the seeds can be infected by wounds and directly; however, wound infection is more rapid. In oil-tea forests, seed wounds are often caused by external factors such as mechanical damage and insects. Symptomatic seeds exhibited brown rot symptoms with irregular, black spots, brown necrosis of the kernels, and accounted for 65% of the surveyed seeds. Rotted seeds were surface-sterilized for 1 min in 75% ethanol, 3 min in 1% sodium hypochlorite, then rinsed for 2 min in sterile water and blotted on dry sterile filter paper. Discolored seed tissues were cut into 3 mm × 3 mm pieces using a sterile scalpel, placed on potato dextrose agar (PDA) medium, and incubated for 7 days at 25°C with a 12-h photoperiod. After 7 days of incubation, circular fungal colonies with dense aerial mycelium produced black, wet spore masses. Four-septate conidia were ellipsoidal to obovoid, measuring 24 (22 to 26) × 6.5 (6 to 7) µm ($n = 30$). Conidia had three median cells, which were dark brown, with a single basal hyaline appendage, 4 (3.5 to 4.5) µm long, and two to four (usually three) apical hyaline appendages, 32 (27 to 35) µm long, similar to these recorded by Crous et al. (2011). Two single-spore isolates cultured

on PDA medium were selected for DNA extraction. The ITS region was amplified using primers ITS5 and ITS4 (White et al. 1990). The partial translation elongation factor 1- α (*tef1- α*) gene region was amplified using primers EF1-728F (O'Donnell et al. 1998) and EF-2 (Carbone and Kohn 1999). The partial β -tubulin (*tub2*) was amplified using primers T1 and Bt2b (Glass and Donaldson 1995). The sequences of ITS (MW391815), *tef1- α* (MW398222), and *tub2* (MW398223) were submitted to GenBank. BLAST analysis demonstrated that these sequences were ~99 to 100% similar to the sequences of ITS (MH553959), *tef1- α* (MH554377), and *tub2* (MH554618) published for *Neopestalotiopsis protearum*. Phylogenetic analysis revealed that all the representative isolates recovered from symptomatic *C. oleifera* seeds showed 91% bootstrap support with *N. protearum* isolate in references. Pathogenicity tests were conducted on 20 healthy seeds. We wounded the seeds by a sterilized needle on the middle position and put 5-mm-diameter agar plugs with actively grown mycelia (strain HNWC04) or pure PDA on the wound. We then covered the wounds with clean masking tape to prevent contamination and desiccation. After inoculation, the seeds were kept at 90 to 100% relative humidity at 25°C in a greenhouse for 3 weeks and monitored daily for lesion development. Twenty days after inoculation, all the seeds inoculated presented similar typical symptoms observed under natural conditions, whereas control seeds showed no symptoms. Koch's postulates were fulfilled by reisolating the same fungus and verifying its colony and morphological characters as *N. protearum*. To our knowledge, this is the first report of *N. protearum* causing oil-tea seed rot in China.

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The author(s) declare no conflict of interest.

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Keywords: *Camellia oleifera*, *Neopestalotiopsis*, phytopathogens

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