Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pestalotiopsis chamaeropsis* Causing Leaf Spot on *Eurya nitida* in China

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Funding: This research was financially supported by the National Natural Science Foundation of China (nos. 31970018, 31360011). Plant Dis. 106:329, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1170-PDN>. Accepted for publication 16 August 2021.

Eurya nitida Korth. (Theaceae) is an evergreen shrub or small tree used as an ornamental tree and nectar source (Khan et al. 1992; Ma et al. 2013). It has high medicinal value for treatment of rheumatoid arthritis, diarrhea, inflammation, ulcer fester, and traumatic hemorrhage (Park et al. 2004). In October 2020, symptoms of leaf spot were observed on *E. nitida* in Meiling Scenic Spot of Nanchang, Jiangxi Province, China (28.78°N, 115.83°E). We surveyed ~300 m² of the mountain area: ~100 *E. nitida* trees scattered near the waterside or planted by the road. Most infected plants were from humid environments, with 15 to 20% disease incidence, and disease severity on a plant basis was 25 to 30%, depending on the field. Sixty infected leaves were collected from 20 individual trees with the same symptoms. Symptoms appeared as tiny circular spots that gradually enlarged into brown circular necrotic lesions and then became a light gray with brown borders and black acervuli at the later stages. Ten leaves randomly selected from the 60 infected leaves were cut into 4-mm² pieces, surface disinfected with 75% ethanol for 30 s and 1% NaClO for 1 min, rinsed three times with sterile water, plated on PDA, and incubated at 25°C in the dark for 5 to 7 days. Five isolates with similar morphological characteristics were obtained. Colonies developed copious white aerial mycelium covering the entire Petri dish area after 7 to 10 days. Conidiogenous cells were discrete, hyaline, and smooth. Conidia were fusiform, ellipsoid, four-euseptate, and 21.86 to 29.80 × 5.95 to 9.80 μm. Apical cells were hyaline with two to three unbranched, tubular apical appendages (mostly three); basal cell was hyaline, obconic with a truncate base; three median cells doliiform to subcylindrical, brown. Morphological characteristics matched *Pestalotiopsis chamaeropsis* Maharachch., K.D. Hyde & Crous (Maharachchikumbura et al. 2014). Two representatives (JAUCC L001-1 and JAUCC L002) were used for molecular identification and verified

based on the amplification of DNA sequences of ITS gene and TEF1-α gene, using the primers ITS4/ITS5 (White et al. 1990) and EF1-526F/EF1-1567R (Rehner and Buckley 2005), respectively. The sequenced loci (ITS: MW845761, MW828589; TEF1-α: MW838967, MZ292464) exhibited >99% homology with *P. chamaeropsis* strain CBS 186.71 in GenBank (KM199326 and KM199473), confirming identification. Phylogenetic reconstruction was generated by using the maximum likelihood method based on the Kimura two-parameter model, with bootstrap nodal support for 1,000 pseudoreplicates in MEGA v. 7.0. Our isolates clustered with *P. chamaeropsis* at 99% bootstrap values. Based on morphological characteristics and molecular phylogenetic analysis, the isolates were identified as *P. chamaeropsis*. Pathogenicity of JAUCC L001-1 was tested indoors by inoculating the top leaves of six healthy *E. nitida* plants. Three plants with three leaves were punctured with flamed needles and sprayed with a 1 × 10⁶ conidia/ml suspension, and another three plants were wound inoculated with mycelial plugs (5 × 5 mm). Mock inoculations were used as controls with sterile water and noninfested PDA plugs on three leaves each. Treated plants were incubated in an artificial climate box with high relative humidity at 25°C. After 10 days, symptoms on all wound-inoculated plants were similar to those previously observed with distinct tiny circular spots; no symptoms appeared on mock-inoculated plants. *P. chamaeropsis* was reisolated from symptomatic tissues but not from mock-inoculated plants; identity was confirmed by morphological characteristics and molecular data, confirming Koch's postulates. *P. chamaeropsis* was previously reported causing leaf blight diseases on *Camellia sinensis* in China (Chen et al. 2020), *Pieris japonica* in Japan (Nozawa et al. 2019), and *Prostanthera rotundifolia* in Australia (Moslemi et al. 2015). This is the first report of *P. chamaeropsis* causing a leaf spot disease on *E. nitida* in China, and it may be more widespread than the sampled location. This finding aids in protection of *E. nitida*, a medicinal and nectar source plant with high economic value.

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The author(s) declare no conflict of interest.

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Keywords: fungi, ornamentals, woody ornamentals, pathogen diversity

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