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NEW DISEASE REPORT

Leaf spot on persimmon caused by *Neopestalotiopsis ellipsospora* in China

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Diospyros kaki, leaf spot, *Neopestalotiopsis ellipsospora*

Persimmon (*Diospyros kaki*) is an important fruit tree and has been cultivated widely for more than 2,000 years (Wang et al., 2018). China is the largest producer of persimmon fruit in the world. During 2019, small brown and black dots were observed on persimmon leaves in an orchard in Yangshuo, Guangxi, China (24°45′ N, 110°24′ E). The infection began as small brown/black dots that expanded and darkened as the disease progressed (Figure 1). Infected leaves were cut into 5 × 5 mm pieces, sterilised with 75% (v/v) alcohol for 10 seconds, 2% (v/v) sodium hypochlorite for one minute, then rinsed three times in sterile distilled water and incubated on potato dextrose agar (PDA) at 25°C under 12/12 hours light/dark for three to five days until hyphae emerged from the diseased tissue. Five isolates with similar morphology were collected and sub-cultured on fresh PDA plates.

After seven days, colonies had spread radially with dense cream to brownish green-aerial hyphae (Figure 2). After two to three weeks, black fruiting bodies were present. Conidia were fusiform to clavate, with five cells and two or three setae, 17.60 to 27.35 × 5.03 to 8.11 μm (mean 21.95 × 6.36 µm, n = 100) in size, the apical and basal cells and setae were colourless, and three median cells were brown, the middle cell being the darkest brown (Figure 3). Morphological characteristics of the colonies and conidia matched the genus description of *Neopestalotiopsis* spp. (Maharachchikumbura et al., 2014). Isolate YSY6-1 was selected as a representative for molecular identification. Primer pairs ITS4/ITS5, BT2a/BT2b and EF1-526F/EF-1567R were used to amplify the DNA fragment of the internal transcribed



FIGURE 1 Leaf spot symptoms on persimmon in the field in Guangxi, China

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FIGURE 2 Colony morphology on potato dextrose agar after incubation at 25°C for seventeen days



FIGURE 3 Conidial morphology of *Neopestalotiopsis ellipsospora* isolate YSY6-1

spacer (ITS), beta-tubulin (TUB2), and translation elongation factor 1 alpha (TEF1-α) (Shu et al., 2020). The sequences of the three loci of YSY6-1 were deposited in GenBank with Accession Nos. OK576920 (ITS), OK594055 (TUB2), and OK594056 (TEF1-α). Based on the combined sequences of ITS, TUB2, and TEF1-α, phylogenetic analysis by neighbour-joining was performed using MEGA10 (version 10.0). The result showed that the YSY6-1 was the most similar to *Neopestalotiopsis ellipsospora* (Figure 4).

A pathogenicity test was done with isolate YSY6-1 on the youngest leaves of two-year-old persimmon seedlings in a greenhouse at 28°C. A 10 µl aliquot of a conidia suspension containing 0.1% Tween 20 (1×10^6 conidia/ml) was used to inoculate each of 11 young leaves per plant, previously damaged by lightly scratching the leaf with a toothpick. In total, three seedlings and 33 leaves were inoculated with YSY6-1 conidia. The control group were damaged and inoculated with sterile

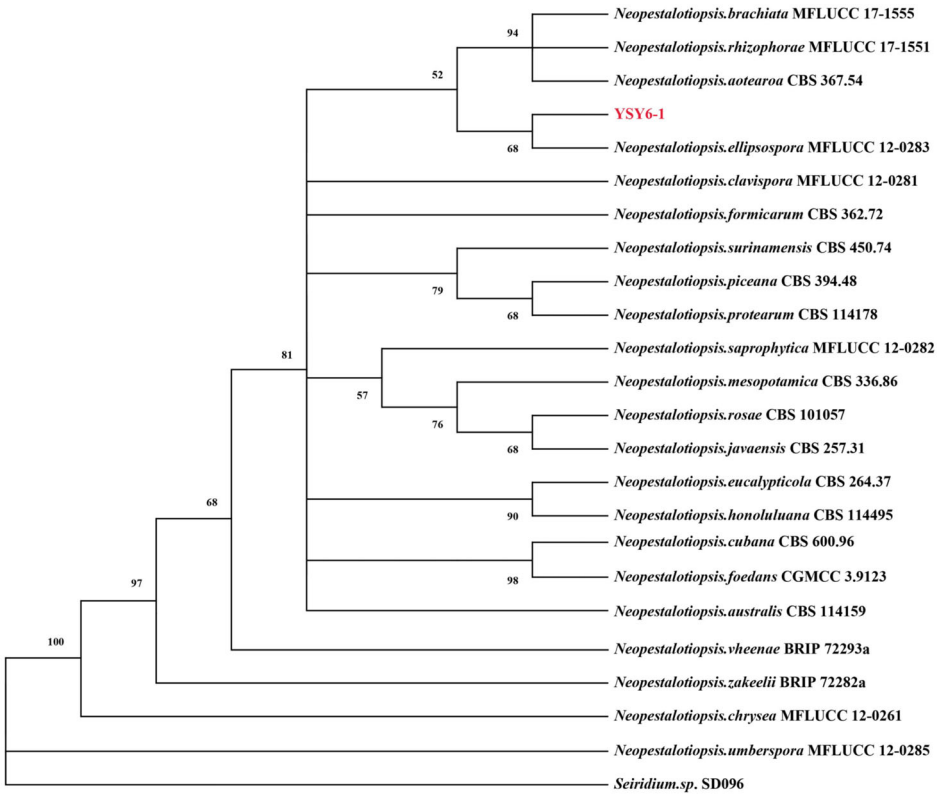


FIGURE 4 Neighbour-joining tree using MEGA10 (version 10.0) inferred from the combined sequences of the internal transcribed spacer, and beta-tubulin and translation elongation factor 1 alpha genes of *Neopestalotiopsis ellipsospora* isolate YSY6-1. Bootstrap values >50% (1,000 replicates) given at the nodes. *Seiridium camelliae* (SD096) was used as an outgroup

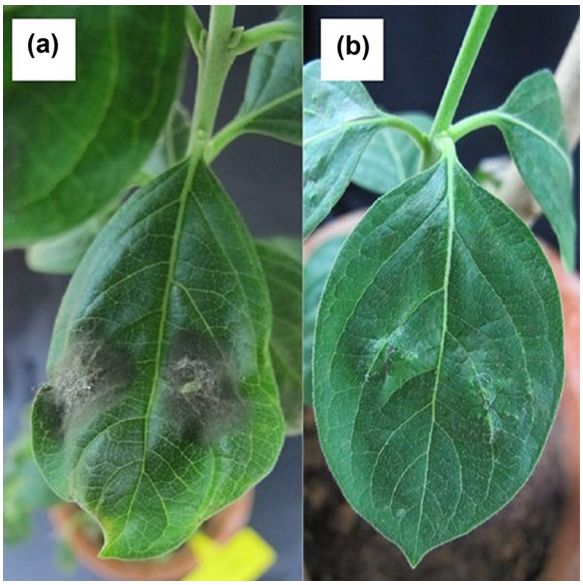


FIGURE 5 Inoculated persimmon leaf with symptoms (a) and uninoculated persimmon leaf (b) five days post-inoculation.

distilled water containing 0.1% Tween 20. All seedlings were covered with plastic bags to maintain high humidity and incubated in the greenhouse at 28°C. Seedlings were sprayed daily with sterile distilled water. After five days, all the inoculated leaves showed symptoms, while no symptoms were observed on the control leaves (Figure 5). The conidia of isolate YSY6-1 did not cause disease on unwounded leaves. Koch's postulates were fulfilled by re-isolating the causal agent from inoculated leaves.

Neopestalotiopsis ellipsospora has previously been reported causing canker on *Acanthopanax divaricatus* in Korea, leaf spot on *Ipomoea batatas* in Guizhou province, China, and as an endophyte in *Diospyros montana* in southern India (Yun et al. 2015; Maharachchikumbura et al., 2016; Reddy et al., 2016). To our knowledge, this is the first report of *N. ellipsospora* causing leaf spot on persimmon in China and worldwide.

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