Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Neopestalotiopsis chrysea* on *Carya illinoensis* in China

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Pecan (*Carya illinoensis*) is important and widely planted nut tree species in Anhui province in China. In a pecan orchard in Anhui, China, 54% of the 1-year-old container-grown seedlings in the nursery developed leaf spots in September 2019. Initially, the brown spots appeared on the leaves. Later, the spots expanded to become brown circles surrounded by a dark brown border. Under severe infection, defoliation occurred, and black acervuli were observed on symptomatic leaves. Disease symptoms were not observed on the fruits. To isolate the pathogen, leaf sections (3 to 4 mm) were excised from the margin of the diseased leaf tissues, surface sterilized in 75% alcohol for 30 s and then in 0.1% mercuric chloride for 30 s, rinsed three times in sterilized distilled water, plated on potato dextrose agar (PDA), and incubated at 25°C in the darkness. Pure cultures were obtained by monospore isolation. The colony of a representative isolate, CZ-4, growing on PDA was circular, white, and cottony, and the surface undulate and pale luteous on PDA. The reverse similar in color. The conidial masses were black and appeared over PDA plates after 14 days. Conidia (15.41 to 29.48 × 4.15 to 7.54 µm) (*n* = 50) were fusiform to ellipsoid and four-septate (one basal and one apical cell hyaline, and three brown median cells), with two to three apical appendages. According to colony and

conidia morphology, the isolates were identified as *Neopestalotiopsis* sp. (Maharachchikumbura et al. 2014). Genomic DNA was extracted from single conidial cultures of representative isolate CZ-4, and the internal transcribed spacer (ITS), translation elongation factor 1- α (*TEF1*), and β -tubulin (*TUB*) genes were amplified with the primers described by Wang et al. (2019). The obtained sequences showed 95 to 99% similarity with those from *Neopestalotiopsis chrysea* accessions in GenBank. The sequences from this isolate were deposited in GenBank under the following accession numbers: ITS, MT459336; *TEF1*, MT469880; and *TUB*, MT469881. A neighbor-joining phylogenetic tree was generated by combining all sequenced loci in MEGA7. The isolate CZ-4 clustered in the *N. chrysea* clade with 98% bootstrap support. To test pathogenicity, 10 detached healthy leaves and ten 1-year-old *C. illinoensis* plants were inoculated with the same pathogens by spraying 50 µl of a conidial suspension (1×10^6 conidia/ml) on both sides of leaves. As a control treatment, 10 additional detached leaves and potted seedlings were inoculated with 50 µl of sterile water. All plants were covered with clear polyethylene bags and incubated in a greenhouse (Center of Co-Innovation, Institute of Forestry) at 23 ± 5°C, 80% relative humidity, and a 12-h light/dark cycle. The experiment was repeated three times. Seven days after inoculation, the symptoms were similar to those on the original infected plants, whereas the control leaves remained symptomless. *N. chrysea* was reisolated from the lesions and morphologically identified, confirming Koch's postulates. To our knowledge, this is the first report of *N. chrysea* associated with leaf spot disease on *C. illinoensis*. This study provides the foundation to further investigate the biology, epidemiology, and management of this disease.

References:

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