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DISEASE NOTES

First Report of *Curvularia lunata* Causing Leaf Blight on Mulberry (*Morus* sp.) in Thailand

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Mulberry (*Morus* L.) is a perennial and heterogeneous outbreeding tree of the Moraceae family. In Thailand, mulberry is cultivated mainly in rural areas where leaves are used to feed silk worms. In August 2013, we first observed leaf blight on the mulberry cv. Buriram 60 in Chiang Mai province (18.79°N, 98.96°E). The infected leaves exhibited brownish to black lesions, starting from the tips or edges of lamina, mainly on younger leaves. Some lesions extended and coalesced, causing leaves to fall as disease progressed. However, this did not cause plant mortality. Four diseased leaves were randomly collected, surface sterilized in 1% NaOCl solution for 3 min, rinsed three times with sterile water, and incubated in a moist chamber to induce sporulation. Single spore isolation (Choi et al. 1999) was performed by preparing conidial suspension in 250 µl of sterile distilled water and dropping on 1.5% (w/v) water agar. After 24 h incubation at room temperature (27 ± 2°C), germinating conidia were individually selected and transferred to half-strength potato dextrose agar (PDA). The single-spore cultures showed various colony morphologies on four different media (Czapex dox, host extract agar, PDA, and Sabouraud dextrose agar). They were downy to cottony, with regular to irregular margins, whitish gray mycelia, later became gray to grayish brown. Conidia (n = 50) on natural substrata were 21.0 to 28.5 (avg. 24.8) µm long, 9.0 to 13.0 (avg. 10.7) µm wide at the broadest part, and similar to conidial sizes on culture media. Conidiophores were erect, unbranched, septate, flexuous in the apical part, with flat and dark brown scars. Conidia were smooth-walled, obovoidal to broadly clavate, brown, 3-septate, with the third cell from the

base conspicuously larger, broader, and darker than other cells, normally curved or occasionally straight, with rounded apical and obconical basal cells. These morphological features described *Curvularia lunata* (Wakker) Boedijn. The fungus is common in soil, on various grasses, cereals, and seeds of other cultivated plants (Ellis 1971). To confirm identification, the internal transcribed spacer (ITS) region of ribosomal DNA of isolate PKC1 was amplified with universal primers ITS4/ITS5 and sequenced directly. A BLAST search of this sequence (GenBank accession no. KX442659) against the NCBI nt/nr database returned a perfect match (100% similarity) with *C. lunata* strain CX-3 (KR633084), the fungal pathogen that caused leaf spot in maize (Liu et al. 2015). Parsimonious tree inferred with PAUP*4.0b10 (Swofford 2002) suggested that *C. lunata* PKC1 was closely related to other sequences of *C. lunata* including the type strain CBS730.96 (HF934911) with 100% bootstrap support. Koch's postulates was conducted to confirm pathogenicity of the fungus. Three drops (10 µl) of a conidial suspension (2 × 10^6 conidia/ml) of the fungus (2-week-old on PDA at 27 ± 2°C) were inoculated onto the third leaves from shoots of 3-month-old mulberry cuttings after surface sterilization of mulberry leaves with 70% ethanol. Sterile distilled water was used as negative control. Inoculated and control leaves were covered with plastic bags for 48 h to maintain high relative humidity. The experiment was repeated twice with four replicates. Identical blight symptoms appeared 7 days after inoculation and reisolation obtained *C. lunata*. The infection assay was conducted on eight mulberry cultivars obtained from The Queen Sirikit Department of Sericulture, and similar symptoms were observed on five of the cultivars (Buriram 51, Buriram 60, Nakhon Ratchasima 60, Sakon Nakhon, and Sisaket 33). Disease severity ratings from 0 to 5 scale (0 = healthy and 5 = severe) showed that cvs. Chiang Mai 60, Kun Pai, and Noi were resistant. Only leaf blight caused by *Alternaria alternata* was reported in Kenya (Peris et al. 2012). This is the first finding of *C. lunata* causing mulberry leaf blight in Thailand, and preliminary screening of resistant cultivars to the disease might be useful for future work.

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