Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Black Spot Caused by *Neoscytalidium dimidiatum* on Sisal in Guangxi, China

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Sisal (*Agave sisalana* Perrine) is an important hard fiber crop that is widely planted in Guangxi, Guangdong, Hainan, Yunnan, and Fujian provinces, China. In July 2019, a new leaf disease of sisal with a disease incidence of about 36% was found in Guangxi. The oval or circular black lesions were 2.3 to 15.9 cm in length and 1.6 to 5.5 cm in width on both sides of the diseased leaves. The central part of the lesions was slightly hollow. The lesions continuously enlarged and ultimately penetrated the leaves. Reddish brown and dark mucus was secreted from the lesions. The junction of lesions and healthy parts was reddish brown to yellow. The diseased leaf fiber and mesophyll tissues were reddish brown and necrotic. Fresh leaf yield was reduced about 30% by the disease, and fiber quality was significantly compromised every year in Guangxi. Six kinds of fungi distinguished by their morphology, size, and color of the colonies were isolated from diseased leaf tissues of 60 sisal plants sampled from five different farms in Guangxi. Isolate JMHB1 was isolated at a rate of 95.67%. The isolate JMHB1 was initially white with dense and hairy aerial mycelium, gradually turning dark gray to olive green on PDA. Conidia, arthrospores, and chlamydospores were observed on PDA in culture. The conidia formed arthric chains, disarticulating, cylindrical-truncate, oblong-obtuse to doliiform, colorless and transparent, zero- to one-septate, and averaging 4.4 to 13.8 × 2.2 to 5.6 μm (*n* = 100). Arthrospores were short columnar, pigmented and transparent, single or formed arthric chains, averaging 5.5 to 17.9 × 2.1 to 3.5 μm (*n* = 100). Chlamydospores were dark brown, round or oval, averaging 4.5 to 9.6 × 4.5 to 8.6 μm (*n* =

100). Pathogenicity testing was conducted by inoculating 3-year-old healthy sisal plants with PDA plugs (5 × 5 mm) on which the fungus had grown for 5 days. Nine healthy plants were wounded on the leaves with a sterile needle, and mycelial plugs were placed on the wounds, covered with sterile moist cotton, and wrapped with Parafilm. Nine control plants were wounded and treated with PDA plugs as the negative control. The test was repeated three times. All treated plants were kept in a greenhouse at ~28°C and 40% relative humidity. After 5 days, only leaves inoculated with isolate JMHB1 showed lesions similar to symptoms observed in the field. The fungus was reisolated from all nine diseased plants, and no symptoms were observed on the leaves of control plants. Molecular identification of the fungus was made by PCR amplification of the internal transcribed spacer (ITS) region of rDNA, EF1-α gene, and β-tubulin gene using primers ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), and TUB2Fd/TUB4Rd (Aveskamp et al. 2009), respectively. The ITS (MT705646), EF1-α (MT733516), and β-tubulin (MT773603) sequences of JMHB1 were similar to the ITS (AY819727), EF1-α (EU144063), and β-tubulin (KF531800) sequences of the epitype of *Neoscytalidium dimidiatum* (CBS 499.66) with 100, 99.65, and 99.02% identity, respectively. Based on pathogenicity testing, morphological characteristics, and molecular identification, the pathogen of sisal causing black spot was identified as *N. dimidiatum* (Penz.) Crous & Slippers (Crous et al. 2006). To our knowledge, this is the first report of black spot caused by *N. dimidiatum* on sisal in China. Sisal is the main economic crop in arid and semiarid areas that is widely planted in several provinces of southern China. The serious occurrence of the disease caused by *N. dimidiatum* has greatly affected the development of the sisal industry and local economic income in China. Identification of the pathogen of the disease is of great significance to guide disease control, increase farmers' income, and promote the development of the sisal industry.

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