Diseases Caused by Fungi and Fungus-Like Organisms

Colletotrichum spaethianum Causing Anthracnose on *Polygonatum cyrtoneura* in Anhui Province, China

Ji Ma,^{1,2} Xiong Xiao,^{1,2} Xingyu Wang,^{1,2} and Min Guo^{1,2,†}

¹ Key Laboratory of Biology and Sustainable Management of Plant Diseases and Pests of Anhui Higher Education Institutes, Anhui Agricultural University, Hefei 230036, China

² College of Plant Protection, Anhui Agricultural University, Hefei 230036, China

J. Ma, X. Xiao, and X. Y. Wang contributed equally to this work.

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Polygonatum cyrtoneura Hua, also known as Huangjing, is an important traditional Chinese herb for treating diabetes and asthma. This plant is commercially planted (approximately 700 ha) in the highland of Anhui Province in China (Liu et al. 2016). In June 2019, brown spots on the leaves of *P. cyrtoneura* were frequently observed in the field in Jinzhai County, Anhui Province. Disease incidence varied from 20 to 30% in a survey site (3 ha) of approximately 25,000 plants. Initially circular or elliptical, reddish colored spots with well-defined brown margin were observed on infected leaves. With time, the spots expanded to large elliptical or shuttle-shaped, pale to dark brown lesions within 10 to 15 days. To identify the pathogen, symptomatic tissues from the lesion margin were excised from 15 infected leaves, surface sterilized with 2% NaClO for 2 min followed by 70% ethanol for 30 s, rinsed three times with sterile water, air dried, plated on potato dextrose agar (PDA) medium supplemented with cefotaxime sodium salt (150 µg/ml), and incubated at 26°C for 3 to 4 days in the dark. Eight morphologically similar fungal isolates were obtained by single sporing. On PDA, colonies were initially white, and they turned pale gray with age. On the reverse side, colonies were initially pale brown and later turned dark black. Conidia were hyaline, curved or slightly curved, aseptate, with a truncate base and acute apex, and measured 14.38 to 20.57 × 2.03 to 5.28 µm ($n = 50$). The appressoria were

solitary, dark brown, ovoid or irregularly shaped, with diameters from 5.61 to 12.43 µm ($n = 50$). These morphological characteristics are consistent with the description of a *Colletotrichum* species (Damm et al. 2009). For molecular identification, the primers ITS4/ITS5, T1/T2, and GDF/GDR were used to amplify the partial region of rDNA-ITS, β -tubulin (*TUB2*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) from fresh mycelia of all eight isolates, respectively. The resulting sequences of ITS, *TUB2*, and *GAPDH* were identical with each other, and sequences from strain PCCS-3 were submitted to GenBank. BLASTn revealed that ITS (MN305792), *TUB2* (MN307740), and *GAPDH* (MN307739) shared 99.8, 100, and 98.8% similarity with the published ITS, *TUB2*, and *GAPDH* sequences from the ex-epitype culture of *Colletotrichum spaethianum* CBS 167.49 (Damm et al. 2009), respectively. To prove Koch's postulates, a pathogenicity test was conducted by spraying 3 ml of conidial suspension of each the eight isolates (10^5 conidia/ml) on 3-month-old healthy *P. cyrtoneura* seedlings (three seedlings per isolate). As a control, three seedlings were sprayed with 3 ml of distilled water. All inoculated plants were maintained in a moist chamber (relative humidity > 90%) at 26°C, with an 8-h photoperiod under fluorescent light. The disease assays were repeated thrice. Brown spots identical to those observed in the field were noted 7 to 10 days after inoculation, whereas control plants remained symptomless. The same pathogen was reisolated and confirmed as *C. spaethianum* by sequencing of ITS, *GAPDH*, and *TUB2*, fulfilling Koch's postulates. Presently, *C. spaethianum* causing leaf spot on *Polygonatum odoratum* was reported from Jilin Province in China (Liu et al. 2020). To our knowledge, this is the first report of *C. spaethianum* infecting *P. cyrtoneura* in Anhui Province, China. Our result expanded the geographic location of *C. spaethianum* as the pathogen on plants belonging to Asparagaceae family in China, and effective control strategies need to be considered to minimize the losses.

References:

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[†]Indicates the corresponding author.
E-mail: kandylemon@163.com (M.G.)