Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Epicoccum sorghinum* Causing Leaf Spot on *Hemerocallis citrina* in China

Y. M. Ma,^{1,2} J. L. Zhou,³ Z. Hu,¹ J. Zhong,^{1,†} and J. Z. Zhu^{1,†}

¹Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Furong District, Changsha City, Hunan Province, 410128, P.R. China

²Jinyun Plant Protective Station, Jinyun County, Lishui City, Zhejiang Province, 321400, P.R. China

³Lishui Cash Crop Station, Lishui City, Zhejiang Province, 321400, P.R. China

Y. M. Ma and J. L. Zhou contributed equally to this work.

Funding: Funding was provided by the Hunan Provincial Innovation Foundation for Postgraduates (CX2018B395), the Key Foundation of Hunan Educational Committee (20A257), and the China Postdoctoral Science Foundation (2018M641562). Plant Dis. 105:2251, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2144-PDN>. Accepted for publication 22 February 2021.

Hemerocallis citrina Baroni, also called yellow flower vegetable (huang hua cai in Chinese), belongs to the family Xanthorrhoeaceae and is widely planted in China, the Korean Peninsula, and Japan for ornamental purposes and vegetable value. In addition, they could also be used as a traditional Chinese medicinal and modern medicinal plant (Du et al. 2014). In August 2019, a leaf spot disease was observed on *H. citrina* plants in Zhejiang Province of China, with approximately 85% incidence in almost 700 ha. Symptoms were first displayed as small, water-soaked, pale chlorotic spots, with yellow halos enlarged into large fusiform spots with brown edges and gray centers. Later, infected leaves were badly damaged and became wilted. Small pieces of infected tissue were excised from the margin of necrotic lesions, surface disinfected with 70% ethanol for 8 s followed by 0.1% HgCl₂ for 1 min, rinsed with sterile distilled water three times, and incubated on potato dextrose agar (PDA, amended with 100 mg/liter of streptomycin sulfate) at 26°C in the dark. Fungal colonies with similar cultural morphology were consistently obtained from repeated isolations. When cultured on PDA, colonies were villous, regular, grayish-green, and turned gray-brown, with the reverse side becoming reddish-brown. Chlamydospores were gray, unicellular or multicellular, nearly spherical, and 11 to 27 × 10 to 23 μm. Pycnidia and conidia were produced on PDA when the fungal colonies were exposed to ultraviolet light for 12 h with a distance of 40 cm from the light source. Pycnidia were

brown, mostly spheroid, and measured 90 to 138 × 120 to 210 μm. Conidia were hyaline, ellipsoidal, unicellular, aseptate, and 4.3 to 5.5 × 1.8 to 2.4 μm. These morphological characteristics agreed with the descriptions of *Epicoccum sorghinum* (Zhou et al. 2018). The DNA of a representative strain HHC6-2 was extracted using the CTAB method, and the rDNA internal transcribed spacer (ITS), actin (ACT), and β-tubulin (TUB) genes were amplified and sequenced, using the primers ITS4/ITS5 (White et al. 1990), ACT512F/ACT783R (Carbone and Kohn 1999), and Bt-1/Bt-2 (Glass and Donaldson 1995), respectively. BLASTn searches of the resulting ITS, ACT, and TUB sequences (accession nos. MW073403, MW080522, and MW080521) revealed 98.58 to 100% identity to the *E. sorghinum* sequences (MT125854, MN956831, and MF987525). The pathogenicity test was carried out by inoculation of potted *H. citrina* plants using conidial suspensions. *H. citrina* seedlings were planted in pots with sterilized soil. Before inoculation, leaves were surface disinfected with 70% ethanol and sterile distilled water. Leaves were inoculated by placing small droplets of conidial suspensions (10⁵ conidia/ml) on one side of the midvein, and three to five drops were used per leaf. Sterile water was used as a control. All the inoculated plants were placed in humid chambers at 25°C for 48 h and then maintained in a greenhouse at 25°C with a 16-h day/8-h night cycle. The pathogenicity assays were performed twice with three replications. Four days after inoculation, yellow to brown spots resembling those observed in the fields developed on the inoculated leaves. However, no symptoms were observed on the controls. *E. sorghinum* was reisolated and identified based on morphological and molecular techniques as described above. To our knowledge, this is the first report of *E. sorghinum* causing leaf spot on *H. citrina*. It seems to be a threat for *H. citrina* planting in China and should be considered in order to reduce losses caused by this disease. This study might provide the basis for diagnosis and control of the disease.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553. <https://doi.org/10.1080/00275514.1999.12061051>
- Du, B., et al. 2014. BMC Complement. Altern. Med. 14:326. <https://doi.org/10.1186/1472-6882-14-326>
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323. <https://doi.org/10.1128/AEM.61.4.1323-1330.1995>
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Zhou, H., et al. 2018. Plant Dis. 102:1039. <https://doi.org/10.1094/PDIS-11-17-1757-PDN>

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fungi, ornamentals, herbaceous/flowering plants, pathogen detection

[†]Indicates the corresponding authors.

J. Zhong; wzzhtx@sina.com, and J. Z. Zhu; 442118535@qq.com