Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Nigrospora hainanensis* on *Oxalis corymbosa* in China

Tao Zheng,¹ Lin Zhao,¹ Meng-Ge Huang,² Jian-Xin Deng,^{1,†} and Yan-Hui Wang^{2,†}

¹Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou 434025, China

²Guangxi Key Laboratory of Biology for Crop Diseases and Insect Pests, Plant Protection Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

Funding: The work was financially supported by National Natural Science Foundation of China (31760522). Plant Dis. 106:1986, 2022; published online as <https://doi.org/10.1094/PDIS-10-21-2149-PDN>. Accepted for publication 12 December 2021.

Oxalis corymbosa DC., introduced to China as an ornamental plant in the mid-19th century, is an important medicinal and edible perennial herb (Zhou et al. 2021). Native to South America, it is an invasive, widely distributed weed found in agricultural farms, gardens, and lawns, especially in the sugarcane fields of Guangxi Province, China. The coverage of *O. corymbosa* in sugarcane fields is normally >70%, and sometimes 100%. In March 2021, a leaf spot disease of *O. corymbosa* from sugarcane fields was encountered in Nanning City, Guangxi. Early symptoms appeared as small, yellowish, round spots. The spots became irregular, usually exhibiting pale brown necrosis in the center with dark brown, necrotic, and well-defined margins. Severely infected leaves became blighted and died. To isolate the pathogen, diseased leaf tissue fragments (4 × 4 mm) were soaked in 75% ethanol for 10 s followed by 2% NaOCl for 1 min and rinsed in sterile water three times. They were transferred to potato dextrose agar (PDA) medium and cultured at 25°C. Pure cultures were obtained by collecting hyphal tips from colonies. The colony features were similar to each other: floccose and white at first, becoming brown, dark brown, or black after 7 days fully covered on PDA in 90-mm Petri dishes. Conidial determination was conducted on synthetic nutrient-poor agar (SNA) medium according to Wang et al. (2017). Conidia were abundantly dispersed on SNA arising from conidiophores, which normally reduced to conidiogenous cells generated from hyphae. The conidiogenous cells were monoblastic, hyaline, globose or ampulliform, and 6 to 8.5 (to 12.5) × 5 to 7.5 (to 9) μm (*n* = 50). Conidia were solitary, smooth, black, spherical or ellipsoidal, and (11 to) 13 to 16.5 × (8 to) 10 to 15.5 μm (*n* = 100). Setae were not observed. The fungus was identified as *Nigrospora* sp. based on the morphology. A representative strain (FSC-3) was selected

for genomic DNA extraction. Sequences of the transcribed spacer region of rDNA (ITS), partial translation elongation factor (*TEF1*), and beta-tubulin fragment (*TUB*) were amplified using primer pairs ITS1/ITS4 (White et al. 1990), EF1-728F and EF2 (Carbone and Kohn 1999; Crous et al. 2013), and Bt2a and Bt2b (Glass and Donaldson 1995), respectively. They were deposited in NCBI GenBank (accession nos. OK083685 [541 bp], OK184809 [481 bp], and OK086377 [421 bp]). BLASTn analysis showed that the ITS, *TEF1*, and *TUB* gene sequences shared 99 to 100% identity with the type strain (CGMCC3.18129) of *Nigrospora hainanensis* (GenBank accession nos. NR153480, KY019415, and KY019464, respectively). A maximum likelihood analysis using the concatenated gene sequences of ITS, *TEF1*, and *TUB* was performed in RAxML v.7.2.8 (Stamatakis 2006) implementing the model of GTRCAT with 1,000 bootstrap replicates. The phylogenetic results indicated that FSC-3 was *N. hainanensis*, which was also confirmed by a morphological comparison with *N. hainanensis* (Wang et al. 2017). Pathogenicity was tested on living *O. corymbosa* leaves (three plants per test) arising from cultivated roots grown for 3 weeks. It was conducted by dropping 5 μl of conidial suspension (10⁵ conidia/ml) on leaves (two sites per leaf) and incubating them in separate containers at 25°C with 90 to 100% relative humidity. Controls were treated with sterile distilled water. Pale brown small spots appeared after 24 h and grew into larger brown spots. Symptoms were similar to field ones, while the control plants remained healthy. The pathogenicity test was repeated twice with similar results. Reisolation of the pathogen from inoculated leaves was determined based on morphology and sequence analysis to fulfill Koch's postulates. *N. hainanensis* had been found in diseased root and leaf tissues of sugarcane in Liuzhou City, Guangxi (Raza et al. 2019). The results indicated that *O. corymbosa* is another host in sugarcane fields in Guangxi. To our knowledge, this is the first report of *N. hainanensis* causing leaf spot on *O. corymbosa* in China.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Crous, P. W., et al. 2013. IMA Fungus 4:133.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Raza, M., et al. 2019. Fungal Divers. 99:1.
- Stamatakis, A. 2006. Bioinformatics 22:2688.
- Wang, M., et al. 2017. Persoonia 39:118.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Zhou, M., et al. 2021. Mitochondrial DNA Part B 6:1138.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: leaf spot, *Nigrospora hainanensis*, *Oxalis corymbosa*, pathogen detection

[†]Indicates the corresponding authors.

J.-X. Deng; djxin555@hotmail.com, and Y.-H. Wang; 214484552@qq.com