Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blight Caused by *Limonomyces roseipellis* on Maize (*Zea mays*) in China

Xi Xu,¹ Xilang Yang,¹ Li Zhang,¹ Hanshui Cao,¹ Peng Cao,¹ Xiangjing Wang,^{1,2} Wensheng Xiang,^{1,2,†} and Junwei Zhao^{1,†}

¹ Key Laboratory of Agricultural Microbiology of Heilongjiang Province, Northeast Agricultural University, Xiangfang District, Harbin 150030, P.R. China

² State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Xi Xu and Xilang Yang contributed equally to this work.

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Maize (*Zea mays* L.) is one of the major crops in China. In July 2020, leaf blight was observed on approximately 18% of maize plants at the 852 Farm, Heilongjiang province. Symptoms appeared as yellow necrotic lesions on leaf tips and margins, which later expanded to the entire leaf. The disease was first observed on the lower leaves of the plants and then progressed up the plant. Thirty symptomatic leaves were collected in 2020, and tissue samples between healthy and necrotic areas (4 × 2 mm) were surface disinfected with 1% NaOCl for 3 min followed by 70% ethanol for 10 s and then washed three times with sterile water. Disinfected tissues were placed on potato dextrose agar (PDA) amended with streptomycin sulfate (50 mg/liter) and incubated at 25°C for 1 week. Cultures were purified using the hyphal-tip technique for morphological and molecular analyses. Morphological characteristics were observed on 1-week-old PDA cultures grown at 25°C. Mycelium changed from cream colored to pale pink or reddish, and the back of the plate turned pink with time. Hyphae were hyaline, 2 to 7 µm wide, with clamps at primary septa. Hyaline basidiospores were spherical to ellipsoid and ranged from 8 to 10 × 6 to 9 µm. Based on the presence of clamp connections and morphological features, the fungus was preliminarily identified as *Limonomyces roseipellis* (Stalpers et al. 1982). To confirm the identity of *L. roseipellis*, primers ITS1/ITS4 (White et al. 1990), MS1/MS2 (White et al. 1990), and LR0R/LR5 (Vilgalys and Hester

1990) were used to amplify the internal transcribed spacer (ITS) region, the partial mitochondrial small subunit rDNA (mtSSU), and the nuclear large subunit rDNA (nuLSU), respectively. These sequences were deposited in GenBank (accession nos. MW067756, MW322806, and MW386178). The ITS sequence had 99.55% nucleotide identity (660/668 bp) with *L. roseipellis* isolate EF82 (GenBank accession no. MK918632). The mtSSU sequence was 99.69% identical (634/636 bp) to that of *L. roseipellis* strain SY-LQG101 (GenBank accession no. KF824718). The nuLSU sequence was 99.14% identical (924/932 bp) to that of *L. roseipellis* (GenBank accession no. EU622844). A single basidiospore was isolated and cultured on PDA for pathogenicity testing. To fulfill Koch's postulates, 10 healthy, surface-disinfected maize plants grown in pots (four- to five-leaf stage) were sprayed with basidiospore suspension (1 × 10⁶ spores/ml); another 10 healthy surface-disinfected maize plants sprayed with distilled water served as controls. Plants were sealed in plastic bags immediately after inoculation and maintained at 90% relative humidity in a mist chamber for 24 h at 25°C with a 12-h light cycle (Nicoli et al. 2016). Plants were moved and maintained in the greenhouse and observed for disease development. The experiment was conducted twice. Leaf blight symptoms appeared on all inoculated plants 3 to 5 days postinoculation and were consistent with symptoms observed in the field. No disease symptoms were observed on control plants. The pathogen was reisolated from diseased plants, and species identification was confirmed by the morphological and molecular method described. *L. roseipellis* has been reported to infect *Cynodon dactylon*, *Lolium perenne*, and *Festuca arundinacea*, respectively. To our knowledge, this is the first report of the identification of *L. roseipellis* as a pathogen of maize in China, and this report will assist with monitoring distribution of the disease to assist with developing management recommendations.

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[†]Indicates the corresponding authors.
W. S. Xiang; xiangwensheng@neau.edu.cn, and
J. W. Zhao; guyan2080@126.com