Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Didymella americana* Causing Corn Stalk Rot in China

Guanghui Hu,¹ Yanyan Zheng,² Chang Liu,¹ Honglei Ren,¹ Jianfei Yang,¹ Shaoxin Hu,¹ Lixin Fu,¹ and Yonggang Li^{2,†}

¹ Institute of Maize Research, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, Heilongjiang, China

² Agricultural College, Northeast Agricultural University, Harbin 150030, P.R. China

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Corn (*Zea mays* L.) is an important food crop and feedstuff worldwide. However, corn stalk rot, caused by multiple pathogens, is globally an economic soil-borne disease. In September 2019, a survey was carried out to characterize pathogenic fungi in corn stalks in Nehe city (48.48°N, 124.88°E), Heilongjiang Province, China. Stalk rot incidence was approximately 5% in three of the fields sampled (5 ha/field). Symptoms included wilting of whole plants, drooping ears, or rapid death of the upper leaves or whole plant from blister stage to physiological maturity (growth stages R2 to R6). A brown to black dry rot or necrosis throughout the central pith and internal tissues of the stalk and crown was observed, which resulted in hollow and soft stalks. Fifteen tissue samples (0.25 cm²) from 15 individual diseased plants were surface disinfested with 75% ethanol for 2 s followed by 0.5% NaOCl for 5 min, rinsed three times in sterile distilled water, and cultured on potato dextrose agar (PDA) with 50 µg/ml of streptomycin at 26°C in darkness. After 3 days, a total of eight fungal isolates with consistent characteristics were obtained from three sampling points and subcultured by transferring hyphal tips onto a new PDA plate. Single-conidium isolates were generated with methods reported previously (Leslie and Summerell 2006). Cultures on PDA were honey to olivaceous buff in the center with dense aerial mycelia and wide buff-colored margins. The dimensions of

conidia from 30-day-old PDA cultures were 4.5 to 15.3 × 1.5 to 4.3 µm (*n* = 50). Often, one to two oil bodies were present within the conidia. Based on these morphological features, the isolates were identified as *Didymella americana* (Aveskamp et al. 2010; Gorny et al. 2016). Genomic DNA was extracted from a representative isolate (YJDA8), and the internal transcribed spacer regions (*ITS*) and translation elongation factor 1-alpha gene (*TEF-1α*) were amplified and sequenced using the primers ITS1/ITS4 (Yin et al. 2012) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. The sequences of YJDA8 (accession nos. MT995077 for *ITS* and MW003707 for *TEF-1α*) showed 99.6% (529/531 bp) and 97.6% (283/290 bp) identity to the sequences of *D. americana* isolate YSGYE6 (accession no. MK945663.1) and isolate K_INSO2_6_10 (MN554764.1), respectively. Pathogenicity tests were conducted by root injection of corn plants at the blister stage in the field. Conidia were obtained from 30-day-old PDA cultures grown at 20°C with a 12-h photoperiod. A conidial suspension (1.5 ml of 1 × 10⁵ conidia/ml) was injected into the base of the maize stems using a 5-ml syringe. For each treatment, five plants were inoculated. Plants injected with 1.5 ml of sterile distilled water served as the control. After inoculation, the plants were managed using conventional methods. All inoculated plants showed symptoms 25 days after inoculation that were similar to those observed in the field, whereas no symptoms were observed on the control plants. The fungus was reisolated and confirmed to be *D. americana*. *D. americana* has previously been reported on corn roots and soybean pods in the United States (Aveskamp et al. 2009 as *Peyronellaea americana*) and on lima bean in Delaware and Maryland (Everts et al. 2020). To our knowledge, this is the first report of *D. americana* causing stalk rot on corn in China. Therefore, its distribution needs to be investigated, monitored, and managed with effective disease management strategies to protect corn.

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[†]Indicates the corresponding author.
E-mail: neaulyg@126.com (Y. G. L.)