Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium cf. longipes* Associated with Maize Stalk Rot in China

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In a field survey from 2017 to 2019, *Fusarium* stalk rot symptoms including discolored, disintegrated stalk pith tissues and lodged plants were observed in maize hybrid lines Fuyu1611, Jidan66, and Danyu8439 grown in fields in Anshan (40°49'39"N, 122°34'6"E), Liaoning province. Its incidence ranged from 15 to 20% and caused a yield loss of up to 30%. Infected pieces of stem tissues were dissected and then sterilized with 1% NaOCl for 1 min, 70% ethanol for 1 min, rinsed three times with sterilized ddH₂O, and dried with filter paper in a hood. Three pieces were placed onto potato dextrose agar (PDA) and incubated at 25°C for 5 days. The colonies were single-spore subcultured on PDA at 25°C for 2 weeks (Leslie and Summerell 2006). Morphological features were observed on PDA and carnation leaf agar (CLA). The average mycelial growth rate was 4.5 to 10.3 mm/day at 25°C on PDA. The colonies produced aerial mycelia, varying from dense white to grayish-rose, and secreted red pigments in the agar. Macroconidia produced on CLA were long and relatively slender, commonly four- to seven-septate, averaging 85.6 × 5.2 μm, with thick walls and pronounced dorsiventral curvature with a distinctly foot-shaped and elongated basal cell and an apical cell that was whip-like. Microconidia were rarely observed on PDA or CLA. Morphological characteristics of the isolates were similar to the features of *Fusarium*

longipes as previously described (Leslie and Summerell 2006). The portions of three phylogenetic loci (EF1-α, RPB1, RPB2) were PCR amplified using the primer pairs EF1/EF2 (O'Donnell et al. 1998), lonR1F/lonR1R (5'-TTTTCCTCACCAAGGAGCAGATCATG-3' and 5'-CCAATGGACTG GGCAGCCAAAACGCC-3') and lonR2F/lonR2R (5'-TATACATTTCCTC CACTCTTTCCCAT-3' and 5'-CGGAGCTTGCCTCCGGTGTGGCCGTTG-3') and sequenced. The consensus sequences were submitted to GenBank (MT513215 and MT997083 for *TEF-1α*; MT513213 and MT997088 for RPB1; MT513214 and MW020572 for RPB2). BLASTn searches indicated that the nucleotide sequences of the three loci of the two isolates shared 94.52 to 99.69% identity with sequences of *F. longipes* strains deposited in the GenBank, *Fusarium*-ID and *Fusarium* MLST databases. A phylogram inferred via maximum likelihood analysis of the combined EF1-α, RPB1, RPB2 partial sequence data of *Fusarium* species was inferred using the CIPRIES website (<https://www.phylo.org>). Isolates LNAS-05-A and LNAS-09-A clustered with *F. longipes*, with 98% bootstrap support. Pathogenicity tests were conducted on three-leaf-stage seedlings and flowering-stage cultivar Zhengdan958 and B104 plants according to previously described methods (Ye et al. 2013; Zhang et al. 2016) with minor modifications. Three days after the roots of the seedlings were inoculated with 1 × 10⁶ macroconidia solution, the leaves and stems exhibited typical wilt symptoms. Twenty flowering-stage maize plants were drilled individually at the second or third node above the soil using an electric drill (Bosch TSR1080-2-Li) to create a hole (8 mm in diameter). An approximately 0.5-ml mycelia plug (125 ml of homogenized hyphal mats + 75 ml of sterilized ddH₂O) was injected into the hole and covered with petroleum jelly. Sterilized PDA plugs were used as a control. The stalk tissue of the split internodes turned dark brown, and the brown area expanded above and below the injection site by 14 dpi. All of the inoculated plants developed characteristic stalk rot symptoms, whereas no symptoms were observed in the controls. The pathogen was reisolated, and its identity was confirmed by sequencing the above-mentioned loci. *F. longipes* was generally regarded as a tropical *Fusarium* species (Leslie and Summerell 2006). This is the first report that *F. cf. longipes* can cause stalk rot of maize under field condition in a temperate, typical corn belt region of China.

References

- Leslie, J. F., and Summerell, B. A., eds. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford, U.K.
- O'Donnell, K., et al. 1998. Proc. Natl. Acad. Sci. U.S.A. 95:2044.
- Ye, J., et al. 2013. Mol. Plant-Microbe Interact. 26:1417.
- Zhang, Y., et al. 2016. PLoS Pathog. 12:e1005485.

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