Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Gaeumannomyces radicicola* Causing Stalk Rot on Maize in Brazil

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Maize (*Zea mays* L.) is an important commodity, and Brazil is the world's second-largest maize exporter. In April 2019, in the second maize crop (*safrinha*), black decayed lesions were observed on roots and wilting of some plants, causing sudden death, in a commercial area in western Paraná state, Brazil. Symptomatic root and stalk tissues were surface disinfected with 70% ethanol for 30 s, 1.5% NaOCl for 1 min, and rinsed three times in sterile distilled water. Slices of necrotic tissues were transferred to PDA and grown for 7 days at 27 ± 1°C with a 12-h photoperiod. Pure cultures were obtained through monospore isolation. Fungal morphology is like *Gaeumannomyces radicicola* (syn. *Phialophora radicicola* var. *radicicola*, *Harpophora radicicola*, *P. zeicola*, *H. zeicola*, and *G. graminis* var. *maydis*) (Hernández-Restrepo et al. 2016). Colonies on PDA were flat, white to light gray, turning gray to black with age. Colony diameter was ~5.2 cm on PDA in the dark after 7 days at 27°C. Conidiophores with slightly thickened wall, mostly branched, varying in dimensions, 57.5 to 166.5 (avg. 128.7) × 2.9 to 5.9 (avg. 4.2) µm, *n* = 25. Conidia were lunate with rounded ends, produced successively at the apex of phialide, 3.3 to 9.7 (avg. 6.6) × 1.5 to 3.6 (avg. 2.5) µm, *n* = 100. Morphological characteristics matched the description of this species (Cain 1952; Gams 2000; McKeen 1952). Total genomic DNA of representative isolate LEMIDPRZm 19-01 was extracted, and the 28S nrDNA LSU, ITS nrDNA including the intervening 5.8S nrDNA (ITS), and partial RPB1 were amplified and sequenced (Hernández-Restrepo et al. 2016; Klaubauf et al. 2014). Primers used were NL1 (O'Donnell 1993)/LR5 (Vilgalys and Hester 1990) (LSU), ITS5/ITS4 (White et al. 1990) (ITS), and RPB1F/RPB1R (Klaubauf et al. 2014) (RPB1). The sequences of LSU (MT123866), ITS (MT114427), and RPB1 (MT123867) were deposited in GenBank and showed 99.67, 99.75, and 100% identity with type material *G. radicicola* CBS 296.53 (KM484962, KM484845, KM485061). Multilocus phylogenetic analysis based on Bayesian inference showed

LEMIDPRZm 19-01 in the *G. radicicola* clade. Ex vivo assays were performed with 5-mm mycelial PDA discs from a 7-day-old culture using detached roots (adapted from Degani et al. 2019) on wounded and unwounded stalks and leaves; each treatment had five replications. PDA discs without fungus were controls. Pathogenicity tests were also conducted in vivo: (i) stalk tissue was inoculated by sterilized toothpick grown on PDA with mycelium and the leaves inoculated as in the ex vivo assay (toothpick without mycelium and PDA discs without fungus were negative controls); (ii) six mycelial PDA discs/500 ml were placed on potato dextrose broth, which was agitated for 10 days to obtain mycelial suspension. Then, 50 ml of suspension was dropped in each 2-liter maize pot with sterilized soil 10 days after emergence. Maize pots with sterilized soil without mycelium were negative controls. Four replications (maize pots) for each treatment were used in both tests. Experiments were repeated twice. In the ex vivo assay, all inoculated tissues with and without wounds showed necrotic lesions. In the first in vivo assay, stalk rot symptoms, including wilting of the inoculated plants causing premature plant death, were observed within 6 days. In the second in vivo assay, inoculated plants had inferior growth compared with the control. Sixty days after inoculation, plants were removed from pots, and root degeneration with symptoms of necrosis was observed. No symptoms were detected in controls, and the pathogen was reisolated from symptomatic tissues, confirming Koch's postulates for all assays. The pathogen was reported in western Paraná state, but it may become a threat to Brazilian maize production. Further monitoring is necessary to better understand the epidemiology of this pathogen to address a strategy for disease control. The pathogen has already been detected in Canada, South Africa, and China. This is the first report of *G. radicicola* in Brazil, as well as in South America.

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