

First report of the blast pathogen, *Pyricularia oryzae*, on *Eragrostis tef* in the United States

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Eragrostis tef (Zucc.) Trotter, commonly known as "teff," is a warm season crop grown primarily in the western U.S. for its versatile, gluten-free grain and is recently gaining wider popularity as a summer forage, and a groundcover for weed exclusion (DeBoer et al. 2017). In late July 2019, leaf lesions, 3-6 mm in diameter, with light colored centers and dark, purple-red edges were observed on leaves of flowering teff plants (cv. 'Dessie') grown as a summer cover at the University of Kentucky Research Farm (UKRF) in Lexington, KY. Following rainfall, the symptoms rapidly progressed to plant death, with 50%-60% of plants in an area of a few hundred square feet being affected. Diseased plants were collected and infected leaves from five plants were placed overnight on a damp paper towel in a Petri dish at 26°C. Conidiophores bearing pyriform, hyaline, bisepate conidia (21.95 to 31.2×8.3 to 10.1 μm ($n = 30$); length of apical cell 5.8 to 9.3 μm ; middle cell: 6.7 to 10.2 μm ; basal cell 8.8 to 13.9 μm) erupted from the lesions. Two single-spore cultures were established (EtKY19-1 and 2) and grown on oatmeal agar medium at 26°C under constant fluorescent light, producing velvety colonies with mid to dark gray pigmentation, and profuse sporulation. Eighteen day old plants of commercial *Eragrostis tef* seeds (Bob's Red Mill, Milwaukie, OR) were spray-inoculated with 10 ml of conidial suspension (10^5 conidia/ml in 0.25% gelatin) until run-off (3 pots/isolate). Inoculations were also performed with isolates from rice (Guy11; Leung et al. 1988), and perennial ryegrass (LpKY97; Farman, 2002) (2 pots ea.) to test for general susceptibility to *P. oryzae*. Controls were sprayed with a 0.25% gelatin suspension. Treated plants were incubated under 16 h light at 27°C/8 h dark at 21°C for up to 10 d. Leaves

inoculated with EtKY19-1 and -2 showed rapid symptom development and shriveled within 5 days post inoculation (Fig S1A). Characteristic diamond-shaped, pale-bordered lesions were observed adjacent to the dead tissues (Fig S1B&C). Abundant conidia were re-isolated from all leaf lesions (Fig S1D), thereby fulfilling Koch's postulates. Mock-inoculated plants, and those inoculated with Guy11 and LpKY97, showed no disease whatsoever after 10 days, showing that *E. tef* is not generally susceptible to *P. oryzae*. The inoculation experiments were repeated twice with the same results.

DNA was extracted from a five-day old culture of monospore isolate EtKY19-1 grown in liquid complete medium (Valent et al. 1986), and a genome sequence was acquired using Illumina technology and assembled using velvet v2.9 (Zerbino and Birney, 2008). A phylogenomic analysis identified EtKY19-1 as being most closely related to *P. oryzae* strain G17 (also from *Eragrostis*), with a sequence divergence of 0.68%, compared with >10% relative to neighbor *Pyricularia* species). The two isolates are preserved in the Farman lab at the University of Kentucky, and the genome assembly, and sequences of widely used diagnostic loci (CAL, CHS, EF-1 α , ACT, BAC6, β t-1, CH7-BAC7, CH7-BAC9, MPG1, and NUT1; Couch et al. 2005) are available at NCBI (PRJNA604303). Given the current interest in increasing teff usage as a summer forage (DeBoer et al. 2017), and considering the severity of disease, and rapidity of symptom progression to plant death, we recommend identifying blast-resistant teff varieties before expanding the acreage planted.

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Figure Legends

Figure S1. Symptoms following inoculation of *Eragrotis tef* plants with fungal isolates from KY. Eighteen day old plants (15 plants/pot x 2 pots) were inoculated with 10 ml of a *P. oryzae* conidial suspensions (10^5 conidia/ml) until run off. The experiment was repeated twice and representative results are shown. Plants were photographed 7 days post inoculation. A) ctrl = 0.25 % gelatin suspension, Guy11 = rice pathogen, LpKY97 = perennial ryegrass pathogen, and EtKY19-1 and EtKY19-2 were two independent, single-spore isolates from diseased *E. tef* in Lexington, KY. B) Leaf lesions caused by EtKY19-1. C) Leaf lesions caused by EtKY19-2; D) Representative images of spores recovered from lesions on inoculated *E. tef* leaves (isolate = EtKY19-1).

