Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pyrenophora teres* f. *teres* Causing Leaf Spot on Wheat in Brazil

R. Garozi,¹ P. Angelotti,¹ H. R. Feksa,² and D. J. Tessmann^{1,†}

¹ Universidade Estadual de Maringá, Maringá 87020-090, Paraná, Brazil

² Fundação Agrária de Pesquisa Agropecuária, Entre-Rios, Guarapuava 85139-400, Paraná, Brazil

Funding: Funding was provided by Fundação Agrária de Pesquisa Agropecuária, Guarapuava, Paraná, Brazil. Plant Dis. 104:3254, 2020; published online as <https://doi.org/10.1094/PDIS-03-20-0570-PDN>. Accepted for publication 7 July 2020.

Pyrenophora teres f. *teres* is known to cause net form net blotch on barley, but pathogenicity on wheat has also been reported in Hungary (Tóth et al. 2008) and east Russia (Mikhailova et al. 2010). The former mentioned symptoms of dark brown necrotic lesions with or without chlorotic margins, whereas the latter described symptoms similar to tan spot. A survey of fungal pathogens causing leaf spot diseases on wheat in the humid subtropical areas of Paraná, Brazil, was conducted in 2016. Twenty-four isolates, from different fields in the municipalities of Guarapuava and Pinhão, were collected from plants at the soft dough stage. The isolates were obtained from leaf fragments of cultivar Toruk and were removed from the edge of lesions resembling tan spot, a major fungal disease of wheat caused by *Pyrenophora tritici-repentis*. The leaves presented oval, diamond, or elongated lesions with tan necrotic spots. They did not always show the brown center, which seemed uncharacteristic of tan spot caused by *P. tritici-repentis*. Yellow halos surrounded the necrotic lesions. Fragments were superficially disinfected for 30 s in 0.5% NaOCl, washed in sterile water, dried with a sterile paper towel, and transferred to Petri plates containing PDA. After 4 days of incubation at 24 ± 2°C and a photoperiod of 12 h, hyphal tips were removed from the PDA and transferred to Petri plates containing V8 medium. Plates were incubated for 14 days in cycles of 12 h at 23 ± 2°C under light and 12 h at 19 ± 2°C in darkness for sporulation. Pure cultures were obtained from single conidia produced on V8 medium. Single-spore isolates, from different fields, were obtained from individual leaves. Conidia measuring 38 to 82 × 16 to 20 µm were smoothly cylindrical and straight, with rounded ends, subhyaline or yellowish brown, with four to seven pseudosepta. Genomic DNA extracted

from cultures grown in yeast extract broth medium was used for PCR amplification of the ITS-5.8S rDNA using primers ITS4 and ITS5 (White et al. 1990). A BLAST search of GenBank revealed that the ITS-5.8S rDNA sequence fragments (505 bp) from 12 of 67 isolates were 99 to 100% similar to *P. teres* (EF452452) (Andrie et al. 2008). DNA sequences of three *P. teres* isolates (20C3, 20A3, and 20A1) were deposited in GenBank (MH594043.1, MH594042.1, and MH594041.1, respectively). They were obtained from different fields in Pinhão (25° 42' 26" S; 51° 38' 0" W). To test the pathogenicity of 20C3, 20A3, and 20A1, a conidial suspension for each isolate with 2 × 10³ to 3 × 10³ conidia/ml was sprayed at the boot stage (BBCH-scale 45) on Toruk leaves and on susceptible barley cultivar Ana 01 plants in pots. Each pot (five to seven plants) was a replicate. Wheat plants were also inoculated with a *P. tritici-repentis* isolate using a conidial suspension at the same concentration as a reference of tan spot symptoms. Wheat and barley plants were also inoculated with sterile water as a control. Six replications were inoculated for each treatment. After inoculation, plants were in a dew chamber for 24 h and then moved to a growth room at 20 ± 2°C and 16-h photoperiod. Initially, leaves of wheat plants inoculated with *P. teres* exhibited small, irregular, dark lesions surrounded by yellow halos; they later exhibited oval to elongated, pale brown, necrotic lesions and yellow halos. Differently from tan spot caused by *P. tritici-repentis*, wheat leaves inoculated with *P. teres* f. *teres* presented a more diffuse yellowing, and the center of necrotic lesions was pale brown. Barley leaves exhibited net form net blotch, so we inferred that the disease was caused by *P. teres* f. *teres* isolates. All inoculated plants presented symptoms, with some variation. *P. teres* f. *teres* was reisolated from the inoculated wheat and barley plants. This is the first report of *P. teres* f. *teres* causing leaf disease on wheat in Brazil. The finding contributes to a better understanding of the etiology of wheat leaf spots in humid subtropical areas of southern Brazil.

References:

- Andrie, R. M., et al. 2008. Fungal Genet. Biol. 45:363.
- Mikhailova, L. A., et al. 2010. Microbiology 79:561.
- Tóth, B., et al. 2008. Plant Pathol. 57:385.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fungi, field crops, cereals and grains, etiology

[†]Indicates the corresponding author.

E-mail: djtessmann@gmail.com (D.J.T.)