Disease Notes

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Bipolaris yamadae* Leaf Spot Disease on Guinea Grass (*Panicum maximum*) in Florida

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Guinea grass is an invasive perennial C4 grass and is a common weed around agricultural crops in Louisiana, Texas, and Hawaii, U.S.A. (Overholt and Franck 2017). In November 2018, leaf spots were observed on Guinea grass occurring in an organic garden located in Gainesville, Florida, U.S.A. Lesions were oblong to irregular, dark gray to brownish center with pale-yellow to brownish black margin. Lesions had coalesced, forming necrotic margins that spread from the leaf tip, resulting in leaf blight and collapse of the canopy. Pieces of symptomatic leaf blades (5 cm²) were surface sterilized (1 min), washed with sterile distilled water, and plated onto water agar medium plates. Plates were incubated at 27°C under 12-h light/dark for 3 to 5 days. Gray to black cottony mycelium was consistent on all plates and produced conidia characteristic of *Bipolaris* spp. Conidia were transferred to potato dextrose agar (PDA) plates with a 0.5-mm-diameter sterile needle. Three isolates (GG1, GG2, and GG3) were successfully grown on PDA. Conidia were black to brown colored, distoseptate with three to eight septa, and measured (60.6 to) 70 to 105 (to 139.8) × (16.0 to) 17 to 23 (to 25.9) µm (average 93.3 µm, *n* = 35, SD = 20.6; average = 21.3 µm, *n* = 35, SD = 2.89). Conidiophores were in groups or single, brown, smooth and straight, septate and swollen at upper tip. Sigma Extract-N-Amp was used for genomic DNA extraction. Primers ITS1/ITS4 and GPD1/GPD2 (Berbee et al. 1999) were used to amplify and sequence the internal transcribed spacer region (ITS) and partial glyceraldehyde-3-phosphate dehydrogenase (*GPDH*) gene, respectively. Sequences were aligned using MUSCLE, and alignment was trimmed for

length. Maximum likelihood phylogenetic trees were constructed with 1,000 bootstrap samples based on the K2+G substitution model, selected by Bayesian information criterion for these two loci using Mega X (Kumar et al. 2018). The ITS and *GPDH* sequences of GG1, GG2, and GG3 (GenBank accessions MT514518 to 20, MT576654 to 56) grouped with *B. yamadae* isolates CPC_28807 and CBS_202.29 in phylogenetic trees (Marin-Felix et al. 2017). All three isolates from Guinea grass were inoculated on Sachs' agar (Luttrell 1958) at 27°C under 12-h light/dark for 1 week, but no sexual morph was observed, and it was consistent for two repeated inoculations. To fulfill Koch's postulates, one isolate, GG1, was used. Conidia were harvested from a 1-week-old colony grown on PDA incubated at 27°C and 12-h light/dark cycle. The concentration of the conidial suspension was adjusted to 10⁵ conidia/ml using a hemocytometer. Using a Paasche H-202S airbrush sprayer, 5-week-old seedlings of Guinea grass were sprayed until runoff with the conidial suspension or 0.5% Tween water only. Each treatment included four replicates, and the experiment was repeated. Leaf spot symptoms were observed on the seedlings inoculated with conidia, whereas seedlings sprayed with water were asymptomatic. Cultures with the expected morphology were isolated from symptomatic leaf blades and absent from control plants. To our knowledge, this is the first report of leaf spot on Guinea grass caused by *B. yamadae* in Florida, U.S.A. *B. yamadae* was previously reported from Guinea grass in India and from other *Panicum* species in the northern United States (Farr and Rossman 2020). *B. yamadae* was also isolated from sugarcane in Cuba and China and from corn in Japan (Manamgoda et al. 2014; Raza et al. 2019), which suggests that it has the potential to impact agronomic crops in Florida, such as sugarcane and corn.

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The author(s) declare no conflict of interest.

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