First Report of Gray Leaf Spot Caused by *Pyricularia oryzae* (synonym: *Magnaporthe oryzae*) in Oat (*Avena sativa*) in Georgia, USA.

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In southeastern U.S., oat (*Avena sativa* L.) is predominantly grown as a grain or forage crop due to its exceptional palatability (Buntin et al. 2009). In November 2020, leaf spot symptoms were observed in an oat field (cv. Horizon 720) in Screven County, Georgia (GPS: 32°38’57.6"N 81°31’32.178"W). Lesions were oblong, whitish to gray in color, and surrounded by dark brown borders. Symptomatic oat leaves were sampled from the field and cut into 1 cm² sections that were surface sterilized, plated onto Potato
Dextrose Agar (PDA) media and incubated in the dark at 23°C. To obtain pure cultures, fungal hyphal tips were transferred onto fresh PDA plates 3 times. The pathogen was identified as *Pyricularia (Magnaporthe)* based on typical conidial morphology (Ellis 1971). Conidia were hyaline, pyriform, 2-septate, and displayed a basal hilum. Conidia measured 5.32 to 10.64 μm (average 8.24 μm) wide by 15.96 to 29.26 μm (average 25.40 μm) long. The identification of *Pyricularia* was further confirmed genetically via PCR amplification followed by sequencing. Genomic DNA was extracted from a 14-day old pure culture using a CTAB method (Doyle and Doyle 1987). The internal transcribed spacer (ITS) region of ribosomal DNA, calmodulin (CaM) gene, and β-tubulin (TUB) gene were amplified using ITS5-ITS4 (White et al. 1990), CMD5-CMD6 (Hong et al. 2005), and Bt2a- Bt2b (Glass and Donaldson 1995) primer sets, respectively. Amplicons were Sanger sequenced and blasted against the NCBI database. Results exhibited 100% (ITS), 100% (CaM), and 99.61% (TUB) homology with *Pyricularia oryzae* Cavara (GenBank accession no. LC554423.1, CP050920.1, and CP050924.1, respectively). The ITS, CaM, and TUB sequences of the isolate were deposited in GenBank as MZ295207, MZ342893, and MZ342894, respectively. In a greenhouse (23°C, 80% RH), Koch’s postulates were carried out by using oat seedlings cv. Horizon 270 grown in Kord sheet pots filled with Sun Gro professional growing mix, and a *P. oryzae* spore suspension containing $10^4$ conidia ml$^{-1}$. The spore suspension (10 ml) was sprayed with an air sprayer onto 7 pots of oat seedlings at the two-leaf stage. Seven supplementary pots of oat seedlings of the same cultivar were sprayed with sterile water to act as controls. After inoculation, plants were covered with black plastic bags that had been sprayed with sterile water to maintain high humidity and incubated
overnight in the greenhouse. The bags were removed the next day, and plants were
evaluated for symptoms in the following days. Seven days after inoculation, plants
displayed symptoms similar to those found in the original field sample. Control plants
showed no symptoms. *Pyricularia oryzae* was consistently re-isolated from inoculated
symptomatic oat tissues. To our knowledge, this is the first report of gray leaf spot
cauised by *P. oryzae* on oat in the state of Georgia and in the continental United States.
*Pyricularia oryzae* can infect several graminaceous plants, including agronomically
important crops such as rice (*Oryza sativa*) and wheat (*Triticum* spp.) (Chung et al.
2020). Phylogenetic analysis on the ITS region using 6 different host lineages was
performed and revealed that this oat isolate was most closely related to the *Lolium*
lineage. This outbreak could have economic implications in oat production.

**References:**

Page 67.


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Figure 1: Gray leaf spot symptoms. Symptoms of gray leaf spot observed on an oat plant taken from the Screven County field, GA (A), lesion of gray leaf spot observed on oat leaf from the Screven County field sample (B); symptoms of gray leaf spot observed in the greenhouse on oat seedlings after 7 days post-inoculation (C); and leaves from control plants showing no symptoms (D).

Figure 2: *Pyricularia oryzae* culture on PDA media after 14 days of incubation at 23°C and *P. oryzae* conidia. Panel A shows the front of a *P. oryzae* culture and panel B shows the back. Pictured in panel C are *P. oryzae* conidia.
Figure 3: UPGMA tree (500 bootstraps) based on ITS sequences of *P. oryzae* MZ295207 from oat (*Avena sativa*) and 6 other reference isolates from different host lineages extracted from GenBank, performed under Mega X (Kumar et. al, 2018).