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First Scientific Report of *Pyricularia oryzae* Causing Gray Leaf Spot Disease on Perennial Ryegrass (*Lolium perenne*) in France

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The fungus *Pyricularia oryzae* causes blast diseases of rice, wheat, and other cereals (e.g., millets). In addition, *P. oryzae* is also responsible for the gray leaf spot disease of ryegrass, a major problem for golf courses in the United States (Farman 2002). A host jump from perennial ryegrass (*Lolium perenne*) onto wheat was recently reported (Farman et al. 2017) and is suspected to be at the origin of strains causing severe epidemics on wheat in South America (Gladieux et al. 2017; Inoue et al. 2017). In 2016, disease on lawns of several football stadiums in France and Switzerland was reported in the nonscientific press. In 2017, *P. oryzae* was said to be the causal agent of this disease in the stadium of Geneva, Switzerland (Visentini 2017). In September 2017, perennial ryegrass plants presenting symptoms of disease were collected in a stadium (stade de la Mosson) in Montpellier, France. After 24- to 48-h incubation on moist filter paper (27°C; 12-h light/dark), diseased symptomatic samples produced spores morphologically similar to *Pyricularia* sp., and four monosporic strains were isolated from leaves and roots and were grown on rice flour agar (RFA) medium. Four ryegrass varieties were grown for 2 weeks in 7 × 7-cm pots filled with peat soil (Neuhaus S, Klasmann-Deilmann, Bourgoin Jallieu, France) in the greenhouse. After inoculation, plants were kept 1 week in a growth chamber (12-h light at 26°C/12-h dark at 21°C; humidity maintained below 70%). Spores were collected from 1-week-old colonies grown on RFA medium (27°C, 12-h light/dark) and used to prepare a spore suspension containing 5 × 10 spores/ml in 0.5% gelatin. Groups of four pots, each containing a different variety, were inoculated by spraying a 10-ml suspension representing a single strain. The negative control consisted of plants sprayed with a solution of 0.5% gelatin and managed as the other treatments

before and after inoculation. Three independent experiments were conducted. All four strains caused symptoms similar to those observed in the original samples and associated with gray leaf spot. Lesions collected from inoculated plants exhibiting symptoms and incubated 24 h in a Petri dish on moist filter paper (27°C; 12-h light/dark) produced typical spores of *P. oryzae*. The DNA of two strains (FR1067 and FR1069) was extracted from 5-day actively growing mycelium in liquid culture (2 yeast extract glucose [2YEG] medium: 10 g/liter of glucose and 2 g/liter of yeast extract), and the genome sequence was produced following published protocols (Gladieux et al. 2017). A phylogenetic analysis was conducted using additional genome sequences of *P. oryzae* strains from different hosts previously used by Gladieux et al. (2017): 10 from *Setaria* spp., 23 from *Oryza sativa*, 3 from *Hordeum vulgare*, 8 from *Eleusine* spp., 2 from *Bromus* spp., 1 from *Avena* sp., 1 from *Eragrostis* sp., 2 from *Brachiaria* spp., 1 from *Festuca* sp., 22 from *Triticum* spp., and 12 from *Lolium* spp. The two French strains from perennial ryegrass were highly similar and clustered in a clade gathering nine out of the 10 strains from *Lolium*. Altogether these results show that the epidemic of gray leaf spot on ryegrass in Montpellier's football stadium was caused by *P. oryzae*. To our knowledge, this is the first scientific report of *P. oryzae* on ryegrass in France. We recommend monitoring the presence of this species on ryegrass because it could be the source of epidemics on wheat, a major crop in France and throughout Europe.

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