Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium meridionale* Causing Stalk Rot of Ryegrass in China

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Members of the *Fusarium graminearum* species complex (FGSC) are the main causal agents of head blight, seedling blight, and stalk rot in wheat and other cereals worldwide. Surveys on species composition and mycotoxin production of FGSC populations have focused on food crops such as wheat, maize, and barley; little is known about the identity of FGSC pathogens in pasture grass. In April 2021, a survey of grass diseases in Hongya County (29.90661°N; 103.37313°E) in Sichuan Province was conducted to understand the etiology of stalk rot in perennial ryegrass (*Lolium perenne*). It was observed in several pastures that about 10% of yield loss in perennial ryegrass was caused by stalk rot. Affected stalks were brown to dark brown and appeared soggy. As infections continued or under conditions of high humidity, some stalks also became flattened. Samples with symptoms of stalk rot or browning of the stem were collected. Symptomatic tissues were cut into ~5-mm segments, surface sterilized in 3% NaOCl solution for 2 min, rinsed three times with sterile distilled water, air dried, plated onto potato dextrose agar (PDA), and incubated in the dark at 28°C. After 3 to 5 days, *Fusarium*-like fungal colonies with reddish-orange mycelium were collected and transferred to new PDA plates for further purification, and purified cultures were obtained by single spore isolation. Four uniform isolates were obtained and their colonies on PDA resembled typical FGSC colonies (Leslie and Summerell 2006; O'Donnell et al. 2004). Colonies had an

average radial growth rate of 8.5 to 11.0 mm/day at 28°C in the dark on PDA. Conidial characteristics were studied on Spezieller Nährstoffarmer agar as described by Wang et al. (2014). Macroconidia were falcate to almost straight, usually with parallel dorsal and ventral lines, 3- to 5-septate, 20.65 to 55.22 (average 39.16) × 2.38 to 6.93 (average 4.42) μm (*n* = 200). No microconidia were observed. The pathogenicity of the isolates was tested on healthy perennial ryegrass (var. Changjiang 1). Two-month-old plants were inoculated by punching a hole in the stem with a sterile toothpick, followed by injection of 20 μl of macroconidia suspension (10⁵ spores/ml). Stems treated with water served as the control. There were 20 plants per treatment. Plants were grown in a growth chamber at 25°C and 90% humidity for 24 h. Stalk tissues at the wound site turned brown after 3 days and the brown area then extended above and below the site. No symptoms were observed in the controls. The same pathogen was reisolated from the infected stems, but not the controls. Thus, the isolated *Fusarium* spp. are a cause of stalk rot in perennial ryegrass based on the fulfillment of Koch's postulates. To identify the *Fusarium* species, portions of the translation elongation factor 1-α (*TEF*) gene sequences from all four strains were amplified and sequenced as described by Wang et al. (2015). The sequences were identical. A sequence of isolate SC1 was submitted to GenBank (no. MZ964308). BLASTn searches of the *TEF* sequence (607 bp) in two databases revealed it had 100% similarity to sequences of *Fusarium meridionale* strain DS27 (no. MN629330) in NCBI and strain NRRL28723 in *FUSARIUM*-ID. A concatenated four-gene phylogeny resolved SC1 and the type specimens of *F. meridionale* (NRRL28723, 29010, and 28436) in a monophyletic clade with 100% bootstrap support, confirming that SC1 belongs to *F. meridionale*. Trichothecene production of strains was evaluated using rice cultures kept at 28°C in the dark for 2 weeks, as described by Desjardins and Proctor (2011). LC-MS/MS analysis indicated that the fungus produced NIV and 4ANIV with average concentrations of 1,400.44 and 3,144.10 μg/kg, respectively. To our knowledge, this is the first report of *F. meridionale* causing disease in perennial ryegrass in China. Further research is necessary to determine its distribution, aggressiveness, and trichothecene production.

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