Diseases Caused by Oomycetes

First Report of Seedling Damping Off in Forage Ryegrass Caused by *Pythium aphanidermatum* and *P. peritum* in Japan

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Italian ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.) are important for hay fields and grazing lands across Japan, with nearly 70,000 ha production, the largest share in forage grass cultivation. In August 2018, damping off of seedlings of both species was observed about 2 weeks after seeding in Tochigi Prefecture, central region of Japan. Roots were brown and decayed drastically with browning of the basal stem. Nearly 90% of the row seedling stands were eradicated in some fields, especially ones seeded from August to early September, when the soil and air temperatures were around 25 to 30°C. Six *Pythium*-like isolates were obtained by isolation from surface-sterilized diseased hypocotyls (1 to 2 cm) placed on water agar. Six isolates were purified as single hyphal tips and deposited at the NARO genebank (https://www.gene.affrc.go.jp/index_en.php), with accession numbers MAFF101946 to 101951. Two of them, MAFF101946 and 101948, were used for detailed study. The isolates were grown in the dark on clarified V8 juice agar for 10 days to produce oogonia. The oogonia of MAFF101946 were globose, colorless, smooth, 21 to 30 µm in size, and had one (to two) antheridia. Oospores were mostly aplerotic, and oogonia walls were 1.1 to 2.3 µm thick. The oogonia of MAFF101948 were globose, colorless, smooth, 19 to 27 µm in size, and had two to five antheridia. Oospores were mostly plerotic, and oogonia walls were 1.7 to 4.2 µm thick. The morphology of the MAFF101946 matched that of *Pythium aphanidermatum* (Edson) Fitzp. (abbreviated PA), and MAFF101948 matched *P. peritum* Drechsler (abbreviated PP) (Van der Plaats-Niterink 1981). DNA sequences of *cox1* (Robideau et al. 2011) and rDNA-ITS regions (White et al. 1990) of each isolate were

analyzed. The *cox1* sequences of the MAFF101946 and 101948 (GenBank accession nos. LC548774 and LC548775) matched 100% (680/680 bp) with those of PA (HQ708486) and PP (HQ708781), respectively. The rDNA-ITS sequence of MAFF101946, LC592700, matched PA (772/777 bp; HQ643439), and MAFF101948 (LC592701) matched PP (HQ643740), with 99% similarity (764/773 bp). The optimal growth temperature was 35°C for both. Their pathogenicity was confirmed by seeding two Italian (cv. Inazuma and Minamiaoba) and perennial (cv. Yatsuyutaka and Natsugoshi-pere) ryegrass cultivars in cell trays containing commercial potting mixture inoculated with the isolates. Barley grains autoclaved with a half volume of water and incubated with each isolate at 25°C for about 2 weeks were added to inoculate the potting mixture (5%, v/v). Separate trays were used for each isolate to avoid cross-contamination; 60 seeds were sown per cultivar, and the trays were placed in a light thermostat chamber under the daily cycle of 16-h light at 30°C and 8-h dark at 25°C. About 3 weeks later, seedlings on the inoculated soil exhibited the symptoms, but not in control (no inoculum) plots. Both inoculated organisms were reisolated from the diseased plants to confirm their pathogenicity. PA was more aggressive with greater percent damping off compared with PP. Both species are known as pathogens of diverse plants including grasses and legumes (Abad et al. 1994; Ao et al. 2018), but to our knowledge, this is the first report of seedling damping off caused by these *Pythium* species in forage ryegrass in Japan. With the increased duration of hot, humid conditions across temperate regions due to global warming, damping off may become a problem in hay fields and pasture, and resistance breeding for these pathogens may be needed.

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