Diseases Caused by Fungi and Fungus-Like Organisms

Sterile White Basidiomycete Fungus *Marasmius graminum*: A New Pathogen Causing Seedling Blight in Rice

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In April 2018, damping-off of rice (*Oryza sativa* L.) seedlings at the two- to three-leaf stage was observed in three fields in the counties of Wharton and Matagorda of Texas and Jefferson-Davis Parish of Louisiana. All affected areas were 1 ha or greater, with 10 to 20% of the seedlings showing the symptoms. Infected seedlings showed dark-brown necrotic lesions on the roots and/or mesocotyls where white superficial mycelium was usually present. Symptomatic tissues excised from 10 diseased seedlings of each field were surface sterilized with 1% NaOCl, double rinsed in sterilized distilled water, and plated on potato dextrose agar (PDA). The plates were incubated at 25°C with a 12-h photoperiod in a growth chamber. After 48 h, hyphal tips of fungal colonies were transferred onto PDA and 12 isolates were obtained. Clamp connections and dolipore septa were observed in young hyphae, indicating that these isolates were a basidiomycete fungus. Young hyphal cells were binucleate based on safranin O stain (Bandoni 1979). No fruiting bodies or sclerotia were produced on PDA after one month of incubation. Based on these morphological characteristics, these isolates were identified as belonging to sterile white basidiomycetes (SWB) (Howard et al. 1977). To further identify the isolates into the species level, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of a representative isolate was sequenced with primers ITS1 and ITS4 (Vinnere et al. 2005). The ITS sequence (GenBank no. MT524457) had more than 97% sequence similarity with known *Marasmius graminum* strains from Denmark (JN943595) (Schoch et al. 2012) and Sweden (MH857692) (Vu et al. 2019). Pathogenicity was tested with three representative isolates in a

growth chamber using a modified method (Carling and Leiner 1990). Pots (6.5 cm in diameter × 7.5 cm in height) were filled with 100 g of sterilized sand and watered to field capacity. Five PDA plugs (4 mm in diameter) from 5-day-old growing culture were placed on the sand surface of each pot. Pots inoculated with PDA plugs without the fungus served as the controls. Five seeds of rice cultivar Presidio were planted into each pot and covered with 10 g of sterilized sand. Pots were maintained at 25 ± 2°C in a growth chamber with a 12-h photoperiod for 14 days. There were four replicated pots for each treatment, and the experiment was repeated twice. After 2 weeks, severe damping-off and associated symptoms similar to those observed in the field appeared in the inoculated pots. No symptoms developed in the control pots. The same fungus was consistently reisolated from infected plants. Based on morphological characteristics and rDNA-ITS sequencing, these isolates were identified as *M. graminum*. The SWB fungus was first reported as a causal agent of stem rot of snap bean in Florida (Howard et al. 1977) and Nebraska (Harveson 2002), root or hypocotyl rot of corn, snap bean, squash, and peanut in Georgia (Bell and Sumner 1984; Sumner et al. 1979), and crown rot of pigeon pea (*Cajanus cajan*) in Puerto Rico (Kaiser et al. 1987). Later, the SWB strain (ATCC 28344) causing stem rot of snap bean in Florida was further identified as *M. graminum* based on nuclear large subunit rRNA gene (Vinnere et al. 2005). Comparing the ITS region of this isolate (AY445120) with our isolate revealed a 99% similarity. To our knowledge, this is the first report that the SWB fungus *M. graminum* causes seedling blight in rice. Identification of this new disease will help to develop management strategies for control of stand loss in rice.

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