Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Lotus Seedpod Withering Caused by *Fusarium proliferatum* on *Nelumbo nucifera* Plants in China

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Nelumbo nucifera (Nymphaeaceae) is well-known in China, and with the increasing value of this crop the planting area is expanding. In May 2019, an unknown withering lotus seedpod was obtained in Guangchang County, Jiangxi Province (26.79°N, 116.31°E). The disease arose between May and July of each year, resulting in the withering and consequent death of ~10% of lotus seedpods, with the disease being most serious during the rainy season. Initial symptoms include the shrinking of young lotus seedpods with concomitant yellowing of the epidermal tissue layer. These pods failed to grow normally and could wither and die within 1 week, with the withering symptoms gradually spreading to associated stem tissues. To characterize the causal pathogens, 10 diseased seedpods were collected and cut into pieces of ~5 × 5 mm, sterilized with 75% ethanol for 30 s, and treated with 0.1% HgCl₂ for 5 min. After being washed four times under sterilized water, samples were then transferred onto PDA and incubated for 7 days at 28°C in the dark. Eight purified isolates yielded large numbers of aerial mycelia that were initially white in color but then changed to a purple-red color over the course of this incubation period. The average mycelial growth rate was 6.3 mm per day ($n = 5$). On PDA, macroconidia exhibited three to five septa and were straight or slightly curved, with a size of 21.6 to 47.4 × 2.5 to 4.6 μm (average: 31.9 × 3.5 μm, $n = 50$). The microconidia were hyaline, ovoid or ellipse, and 4.6 to 13.5 × 2.2 to 4.3 μm (average: 8.7 × 3.1 μm, $n = 50$). The morphological features of these fungi were noted to be in line with those of *Fusarium proliferatum* (Leslie and Summerell 2006; Zhao et al. 2019). To confirm identity at the molecular level, the universal ITS4/ITS5 primers

(White et al. 1990), the *Fusarium*-specific pair PRO1/PRO2 (Mulè et al. 2004), EF1T/EF2T (O'Donnell et al. 1998), and RPB2F/R (O'Donnell et al. 2010) primers were utilized to amplify the internal transcribed spacer 1 (ITS1)–5.8S rRNA gene–internal transcribed spacer 2 (ITS2), calmodulin, alpha elongation factor genes, and RNA-dependent DNA polymerase II subunit from these isolates. Following alignment of the resultant sequences with GenBank via a BLAST analysis, the sequences (MW862499, MW762531, MW767988, and MW831311, respectively) showed 100% identities to the corresponding DNA sequences in *F. proliferatum* (MW817705, LS423443, MH153750, and MW091308, respectively). Based upon these morphological and molecular findings, this pathogen was identified as *F. proliferatum*. Pathogenicity testing was then performed using five plump healthy lotus seedpods. Sterile needles were used to generate wounds (2 mm deep, 1 mm in diameter), and a 10-μl suspension of prepared spores (1.0×10^6 spores/ml) derived from a 7-day-old culture grown on PDA was injected into the wound sites of the lotus seedpod. As a control, five seedpods were additionally wounded and injected with 10 μl of sterile water. The experiments were repeated three times with five biological replicates. All seedpods were then incubated at 28°C in a growth chamber (12 h light/dark) with 80% relative humidity. After a 3-day incubation period, wounded sites injected with spore suspensions exhibited browning. Following a 5-day incubation period, a mean lesion diameter of 9.8 mm was observed, with white mycelia growing on the wound surface and with evident withering of the internal and external tissues near the wound site. In contrast, control wound sites remained healthy. We were again able to isolate *F. proliferatum* from the infected lotus seedpods. Finally, eight isolates were obtained and were identified as the pathogen based on these morphological and molecular analyses, thus fulfilling Koch's postulates. This is the first report to our knowledge to have described a case of *F. proliferatum* causing lotus seedpod withering in China, providing a foundation for future research efforts aimed at preventing diseases caused by this pathogen.

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