Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium oxysporum* Associated with Stem Rot on Seedlings of Jinxianlian (*Anoetochilus roxburghii*) in China

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Anoetochilus roxburghii is an important Chinese herbal medicine plant in the Orchidaceae known as Jinxianlian. This orchid is cultivated and mostly adopted to treat diabetes and hepatitis. About two billion artificially cultivated seedlings of Jinxianlian are required each year, and approximately \$600 million in fresh *A. roxburghii* seedlings are produced in China. Since 2011, sporadic occurrences of stem rot on Jinxianlian have been observed in greenhouses in Jinhua City (N 29°05', E 119°38'), Zhejiang Province. In 2018, nearly 30% of seedlings of Jinxianlian grown in greenhouse conditions were affected by stem rot in Jinhua City. Symptoms initially occurred in the stem at the soil line causing dark discolored lesions, rotted tissues, wilting, and eventually plant death. A total of 23 diseased seedlings collected from seven different greenhouses were surface sterilized with 1.5% sodium hypochlorite for 3 min and rinsed in water. Pieces of disinfected tissues from each sample were plated on 2% potato dextrose agar (PDA) and incubated at 25°C in the dark for 5 days (Kirk et al. 2008). A total of 19 isolates were recovered. They developed colonies with purple mycelia and beige or orange colors after 7 days of incubation at 25°C on PDA and carnation leaf agar (CLA) media (Kirk et al. 2008; Zhang et al. 2016). Colonies on PDA had an average radial growth rate of 3.1 to 4.0 mm/day at 25°C. The colony surface was pale vinaceous and floccose with abundant aerial mycelium. On CLA, aerial mycelium was sparse with abundant bright orange sporodochia forming on the carnation leaves.

Microconidia were hyaline and oval ellipsoid to cylindrical (3.7 to 9.3 × 1.3 to 2.9 μm) (*n* = 19). Macroconidia were three to five septate and fusoid subulate with a pedicellate base (27.4 to 35.6 × 3.2 to 4.2 μm) (*n* = 19). These morphological features were consistent with *Fusarium oxysporum* (Lombard et al. 2019; Sun et al. 2018). To confirm the identification, the internal transcribed spacer region (ITS) and translation elongation factor1 (TEF) were amplified from the DNA of three of the isolates chosen at random using the primer sets ITS1/ITS4 and EF1/EF2 (Lombard et al. 2019; Sun et al. 2018), respectively. BLAST analysis revealed that the ITS sequences (OK147619, OK147620, and OK147621) had 99% identity to that of *F. oxysporum* isolate JF2 (GenBank MN626452), and the TEF sequences (OK155999, OK156000, and OK156001) had 100% identity to that of *F. oxysporum* isolate gss100 (GenBank MH341210). A multilocus phylogenetic analysis by Bayesian inference and maximum likelihood trees based on ITS and TEF indicated that the pathogen grouped consistently with *F. oxysporum*. Three isolates were chosen at random to evaluate pathogenicity. Uninfected healthy *A. roxburghii* seedlings about 40-days-old planted in sterilized substrates were sprayed with distilled water containing 2 × 10⁶ conidia/ml suspensions as inoculums, and plants sprayed with distilled water alone served as controls. Plants were incubated at 25°C and 85% relative humidity. Ten plants were inoculated for each isolate. After 10 days, all inoculated plants developed stem rot symptoms, while control plants remained healthy. Cultures of *Fusarium* spp. were reisolated only from inoculated plants with a frequency of 100% and reidentified by morphological characteristics as *F. oxysporum*, fulfilling Koch's postulates. To the best of our knowledge, this is the first report of *F. oxysporum* causing stem rot on *A. roxburghii* seedlings. As *F. oxysporum* is a devastating pathogenic fungus with a broad host range, measures should be taken in advance to manage stem rot of *A. roxburghii*.

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