Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of Leaf Spot Caused by *Neodeightonia phoenicum* on Pygmy Date Palm (*Phoenix roebelenii*) in China

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The pygmy date palm (*Phoenix roebelenii*) is a popular ornamental plant widely cultivated in tropical regions as well as in China. In June 2018, new leaf spot symptoms were observed on *P. roebelenii* in several different parks in Zhanjiang City of China. The early symptoms of infected leaves were small, round, pale brown spots. As the size of these spots increased, they coalesced to form larger irregular necrotic lesions surrounded by dark brown edges, which eventually led to leaf wilting and defoliation. A filamentous fungus was consistently isolated from infected leaf samples. Colonies on PDA at 25°C (12 h light/dark) were initially white with abundant aerial mycelium, which turned fluffy and dark olivaceous after 1-week culture. Pycnidial conidiomata were black and globose and formed on pine needles in water agar at 25°C (12 h light/dark) after 21 days. Conidiogenous cells were hyaline, cylindrical, and holoblastic. The conidia were ovoid to ellipsoid, thick walled, initially hyaline and aseptate, and later turned dark brown and 1-septate with a striate appearance to conidia, being 11.6 to 25.0 × 9.6 to 12.0 µm (average 20.4 × 10.1 µm). For molecular identification, the partial sequences of internal transcribed spacer (ITS) regions, translation elongation factor (EF-1α), and β-tubulin (TUB) genes of two representative isolates (RYCK-1 and RYCK-2) were amplified and sequenced using primer pairs ITS/ITS4 (White et al. 1990), EF-688F/EF-986R (Carbone and Kohn 1999), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The sequences of

the above three loci of the two isolates (accession nos. ITS, OK329968 and OK329969; EF-1α, OK338067 and OK338068; TUB, OK338069 and OK338070) showed 98.4 to 100.0% identity with the existing sequences of ex-type culture CBS 122528 of *N. phoenicum*. A multilocus phylogenetic analysis of the three loci concatenated sequences using the maximum likelihood method showed that the isolates belonged to *Neodeightonia phoenicum*. Based on the morphological characteristics and molecular analysis of the isolates, the fungus was identified as *N. phoenicum* (Phillips et al. 2008). To confirm pathogenicity, five 1-year-old potted plants were used for each isolate (RYCK-1 and RYCK-2), and the plants were inoculated by pricking the epidermis of the leaf with a needle. Five leaves of each plant were sprayed with 100 µl of a conidial suspension (1 × 10⁶ conidia/ml) to the wounded surface for each plant. Sterilized distilled water was used as the control and the experiment was repeated. All the plants were incubated at 26 ± 2°C (12 h light/dark) and covered with plastic bags to maintain constant high humidity. After 14 days, all the inoculated leaves showed the same symptoms as those observed in the original diseased plants, but the control plants remained healthy. The reisolated fungus was identified as *N. phoenicum* by morphological and molecular characteristics. *N. phoenicum* is an important pathogen of *Phoenix* species plants worldwide and has been reported to cause shoot blights and stalk rots on *P. dactylifera* and *P. canariensis* in Greece (Ligoxigakis et al. 2013) and root rot on *P. dactylifera* in Qatar (Nishad and Ahmed 2020). To our knowledge, this is first report of *N. phoenicum* causing leaf spot on *P. roebelenii* in China.

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