Diseases Caused by Fungi and Fungus-Like Organisms

First Record of *Paramyothecium roridum* Causing Leaf Spot on *Physostegia virginiana* in China

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Physostegia virginiana Benth. (false dragon head flower), belonging to the family Lamiaceae, is a perennial plant and is usually used as landscape plant in parks and wetlands in China. It is also widely used as an ornamental plant for cut flowers all over the world (Cardin et al. 2002). In June 2019, leaf spot symptoms were observed on *P. virginiana* in Zibo botanical garden of Shandong Province, China (36.79°N, 118.02°E). We surveyed about 200 m² of planting area, and most of the infected plants were close to the water or wet places, with ~20% disease incidence, which was concentrated in the lower leaves of plants. The symptoms that appeared on leaves were mostly round or oval spots, light to dark brown in color, and 3 to 8 mm in diameter. Severe leaf lesions were linked together, causing early fall of leaves. Small pieces of 15 infected leaves were collected to confirm the causal pathogen. The samples were sterilized by 70% ethanol for 30 s followed by 5% NaClO for 1 min, rinsed in sterile water three times, plated on potato dextrose agar (PDA), and incubated at 25°C in the dark for 7 days (Zhu et al. 2013), and seven isolates were obtained from 10 diseased samples. The stroma of fungal mycelium was initially white, gradually turning dark green to black, and the margin of the colony was regular, with concentric rings that were black sporodochia aggregates. Conidiophore hyaline, producing second- to third-order complex branches, arising as three to four conidiogenous cells from the tip of the branches, conidiogenous cells ampulliform to cylindrical. Conidia were aseptate, unicellular, hyaline, cylindrical, and their dimensions varied from 4.8 to

8.2 × 1.7 to 2.4 µm with rounded tips. The morphological characteristics of the isolates matched features described for *Paramyothecium roridum* (Tode) L. Lombard & Crous, *comb. nov.* (Lombard et al. 2016). For molecular identification, genomic DNA was extracted from five representative single-spore isolates. The partial coding genes of internal transcribed spacer (ITS) and calmodulin (*cmdA*) from the original isolates were amplified with primers ITS1/ITS4 and CALDF1/CALDR1 (Lawrence et al. 2013; White et al. 1990), respectively. The sequenced genes (GenBank accession nos. MT318535 and MT454826) exhibited 98.71 and 100.00% homology with type specimen of *P. roridum* strain CBS372.50 (GenBank accession nos. MH856665.1 and KU846271.1), respectively, confirming the morphological identification. Pathogenicity of the fungus was tested indoor by inoculating five living, healthy *P. virginiana* plants with three leaves, which were inoculated with 10 µl of conidial suspension (2 × 10⁵ conidia/ml) from a 10-day-old culture on PDA, and five other inoculated plants with 10 µl of sterile water served as controls. Treated plants with the inoculated leaves were covered by plastic bags in the greenhouse with 14-h light/10-h dark with ~80% relative humidity at 25°C. As time went by (about 3 to 7 days), on the leaves inoculated with conidial suspension appeared similar symptoms as described above, whereas negative controls were still healthy. The same pathogens were isolated from the diseased leaves and repeated three times with same results as those that were obtained previously from the outdoor plants, including morphological and molecular results, which confirmed Koch's postulates. To our knowledge, this is the first record of *P. roridum* causing leaf spot on *P. virginiana* in China. The finding is beneficial to the better application of *P. virginiana*, a very common ornamental plant.

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