



First report of *Diaporthe foeniculina* causing yellowing and dieback on *Ficus benjamina*

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Received: 27 Jan 2020. Published: 26 Feb 2020.

Ficus benjamina, commonly called weeping fig or Benjamin fig, is a flowering plant in the family *Moraceae*. It is widely grown as an ornamental tree or hedge. During the spring of 2019, yellowing and dieback of *F. benjamina* were observed in different regions of Hamedan Province, Iran (Fig. 1).

To isolate the causal organism from infected tissues, small twig pieces were cut from the boundary between diseased and healthy tissue, surface-sterilised for one minute in 1% (w/v) sodium hypochlorite, rinsed three times with sterile water, dried on sterile filter paper, transferred to potato dextrose agar (PDA; Merck, Darmstadt, Germany) and incubated at 25°C in the dark. Five to seven days after incubation, fungal isolates were obtained from symptomatic tissues. The morphological characteristics were determined based on pure cultures grown on PDA. Colony morphology, size and shape of 30 conidia and conidiophores were recorded after seven days incubation. Colonies on PDA were slow-growing and white with sparse aerial mycelium (Fig. 2). After 15 days, conidioma (pycnidium) production was observed (Fig. 3). Microscopic identification was done using a Leica DMLB microscope. Pycnidia globose to subglobose with an elongated and black neck. Conidiophores hyaline, sub-cylindrical and cylindrical, filiform, branched above the septa, tapering towards the apex, 1-3 septate, (19-)20-28(-32) × 2(-3) µm. Conidiogenous cells hyaline, subcylindrical and filiform, straight, slightly tapering towards the apex, collarette not flared, with prominent periclinal thickening, (10-)11-15(-17) × 2(-3) µm. Alpha conidia aseptate, hyaline, smooth, ellipsoidal or fusiform and 8-9 × 2-2.5 µm (Fig. 4a). Beta conidia aseptate, filiform, slightly curved and 25-29 × 1-1.5 µm (Fig. 4b). Based on morphological and cultural characteristics, the isolates were identified as *Diaporthe foeniculina* (Sacc.) Udayanga & Castl. (= *Phomopsis foeniculina* (Sacc.) Udayanga & Castl) (Udayanga *et al.*, 2014). Representative cultures were deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRAN3161C).

In order to confirm Koch's postulates, pathogenicity tests were performed on healthy seedlings in laboratory conditions at 25°C with natural daylight as follows. Stems were wounded using a sterile 5 mm diameter metal cork borer. A mycelial plug (5 mm diameter) obtained from the margin of a

seven-day-old fungal colony (IRAN 3161C) was placed in the wound. The wound was wrapped with plastic film and for the control plants, a plug of clean PDA was placed in the wound. Two weeks after inoculation, symptoms were observed on the inoculated plants while no symptom appeared on the control. *Diaporthe foeniculina* was consistently reisolated from artificially infected plants (Fig. 5).

To confirm the morphological identification, one isolate (IRAN 3161C) was selected for molecular analysis. Genomic DNA was extracted from mycelium of a pure culture as described by Sharma *et al.* (2002). Molecular identification was performed based on the beta-tubulin gene which was sequenced using primers BT2A and BT2B (Glass & Donaldson, 1995). BLAST search of a 566 bp sequence (GenBank Accession No. MG367462) showed 100% identity with *D. foeniculina* (KC843213). These test results confirmed *D. foeniculina* to be the causal agent of the newly observed disease on *F. benjamina*. To our knowledge, this is the first report of yellowing and dieback caused by *D. foeniculina* on *F. benjamina* (Farr & Rossman, 2020).

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Figure 1



Figure 2



Figure 3

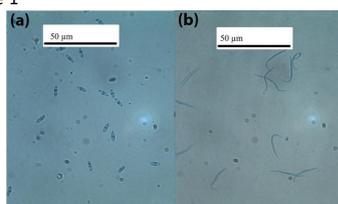


Figure 4



Figure 5

To cite this report: Esmaeilzadeh A, Zafari D, Bagherabadi S, 2020. First report of *Diaporthe foeniculina* causing yellowing and dieback on *Ficus benjamina*. *New Disease Reports* 41, 16. <http://dx.doi.org/10.5197/j.2044-0588.2020.041.016>

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