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First report of *Diaporthe arecae* causing leaf spot on *Cenostigma tocantinum* in Brazil. Primeiro relato de *Diaporthe arecae* causando mancha foliar em *Cenostigma tocantinum* no Brasil

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The forest species popularly known as “pau-pretinho” (*Cenostigma tocantinum* Ducke) is one of the most used in urban arborization of the municipality of Manaus, Amazonas (Brazil). It is a native species to the Amazon and has low susceptibility to attack by pests and pathogens (GARCIA; MORAES; LIMA 2009). Throughout the year 2018, symptoms were observed on the leaves positioned in the lower third of the crown of *C. tocantinum* trees. The symptoms were characterized by areas of light brown color in the center and dark brown edges observed on leaves. The symptomatic trees were located on campus of Federal University of Amazonas (UFAM), in Manaus, where the temperature and rainfall ranged from 24°C to 33°C, and 113 mm to 335 mm in this period. Small fragments (2 to 3 mm) of symptomatic leaf tissues were surface disinfested, plated on potato dextrose agar (PDA) amended with 0,25 $g.L^{-1}$ of chloramphenicol, incubated at 25°C and hyphal tips were transferred to fresh PDA plates. The monoculture mycelium was initially white, gradually darkening and becoming dark brown after 14 days (Figure 1). No presence of conidia in PDA culture medium was observed after 20 days. The pathogenicity test was conducted on *C. tocantinum* seedlings. Leaves were inoculated with mycelial discs. After inoculation the plants were sprinkled with the mixture of water and fungal mycelium and kept in plastic bags to maintain moisture. The plant control was inoculated with agar disc, sprayed with water, and kept in plastic bags. Symptoms similar to those observed in the naturally infected plant were observed in the *C. tocantinum* seedlings after 15 days (Figure 2). The symptomatic leaves were placed in a humid chamber and after 48 h spores (measures) were observed at the apex of the conidiomas. The pathogen was reisolated from the lesions, and their identity has been performed by molecular methods. The 3 Kbp ribosomal (SSU, ITS and LSU) and beta-tubulin (TUB2) regions (acession number: MK299422, MN067841), were amplified and sequenced using primers NS1-NS8, ITS1, ITS4, Uni-R primers and T1-T2 (FELL, 1993; O’DONNELL; CIGELNIK,

1997; TEMPLETON et al., 1992; WHITE et al., 1990). Concatenated phylogenetic analyses based on maximum parsimony (MP) analyses using MEGA7 with our sequences and reference isolates of *Diaporthe* spp. (DISSANAYAKE et al., 2017) showed high similarity with the sequence of *Diaporthe arecae* (CBS 161.64). To the best of our knowledge this is the first report of *Diaporthe arecae* causing leaf spot on *C. tocantinum* in Brazil.

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Figure 1. Colony of *Diaporthe arecae* with 14 days on PDA medium.

Figure 2. Symptoms of *Diaporthe arecae* caused by artificial inoculation on *Cenostigma tocantinum* seedlings.

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