Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Choanephora cucurbitarum* Causing Leaf Wilt, Flower Rot, and Stem Necrosis on *Crotalaria breviflora*

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Crotalaria breviflora (Fabaceae) is used as green manure crop because of its nitrogen fixation and nematode control (Nascimento et al. 2020). In April 2018, leaf wilting, flower rot, and stem necrosis symptoms were observed on *C. breviflora* with 100% incidence, in Sorriso (12° 33' 31" S, 55° 42' 51" W), Santa Carmem (11° 55' 52" S, 55° 16' 47" W), and Sapezal (12° 59' 22" S, 58° 45' 52" W) counties in the state of Mato Grosso, Brazil. Three monospore isolates were isolated from symptomatic leaves, cultivated in potato dextrose agar (PDA) medium, and deposited at the Cultures Collection of the University of Brasília (codes CCUB 1293, CCUB 1667, and CCUB 1668). Colonies on PDA were white and cottony with presence of hyaline and coenocytic hyphae. The mycelia later became pale yellow with abundant reproductive structures. Sporangiophores were hyaline, aseptate, unbranched, and apically dilated to form a clavate vesicle, which produced secondary vesicles bearing sporangia. Secondary vesicles were clavate, light brown, and 37 to 51 µm in diameter. Sporangia were brown to dark brown, globular to ellipsoid, 115 to 140 µm long, and 96 to 122 µm wide. Sporangiospores ($n = 30$) were brown to reddish-brown, ellipsoid to ovoid, with longitudinal striae, 14 to 19 µm long, and 8 to 12 µm wide. Some had hyaline appendages

at both ends. Their morphological characteristics were consistent with the descriptions of *Choanephora cucurbitarum* (Kirk 1984). To confirm the identity, the DNA of the three isolates was extracted, and the sequences of small subunit (SSU), large subunit (LSU), and complete internal transcribed spacer (ITS) of rDNA were amplified using V9G, ITS3, and LR5 primers (GenBank nos. MN897836, MN897837, and MN897838). The sequences were aligned with MAFFT software. The alignment matrix was subjected to maximum likelihood analysis using RAxML version 8, and Bayesian inference was performed in MrBayes version 3.1.2. The tree was edited in FigTree software. The sequences showed 100% identity with the sequences from *C. cucurbitarum* found in GenBank. To confirm pathogenicity, a suspension at 5.4×10^6 spores/ml was prepared from a 15-day-old culture grown at 25°C and sprayed on asymptomatic plants of *C. breviflora*. Sterilized water was sprayed as the control. Plants were kept in a humid chamber at 20°C for 48 h. Initial symptoms were visualized 16 days after inoculation. Complete necrosis of leaves and stems with spore mass on infected tissue was observed 19 days after inoculation. To satisfy Koch's postulates, the fungus was successfully reisolated from the infected tissues. No symptoms were observed on the control plants. In Brazil, this pathogen has been reported on *Brassica oleracea* var. *capitata*, *Capsicum annuum*, *Crotalaria spectabilis*, *Cucurbita* sp., and *Vigna unguiculata* (Alfenas et al. 2018; Mendes and Urben 2020). *C. cucurbitarum* has been reported to have a wide range of hosts (Farr and Rossman 2020). It can infect the crops grown in rotation or in succession, including common bean, corn, cotton, quinoa, soybean, and sunflower. Therefore, this pathogen is of epidemiological importance and poses a threat to croplands where environmental conditions are conducive to disease development and spread. To our knowledge, this is the first report of *C. cucurbitarum* causing leaf and flower wilt and stem rot on *C. breviflora* in the world.

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