Dichotomophthora sp. causing leaf spot and foliar abscission on *Anredera cordifolia* in Brazil

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Abstract *Dichotomophthora* sp. is described as causing leaf spot and early foliar abscission on *Anredera cordifolia* in Brazil. Morphologically, the fungus fits with *D. lutea*, however, ITS sequence showed high homology with *Bipolaris* sp. recorded on *Basella alba* in Florida and with *D. portulacae* recorded on *B. rubra* in China. Additional studies are needed to clarify the taxonomic status of *Dichotomophthora* species on members of Basellaceae.

Keywords Basellaceae · Dematiaceous hyphomycete · ITS · Tropical fungi

Anredera cordifolia (Basellaceae), also known as madeira or mignonette vine, is a highly invasive species in Australia and in some Pacific Islands (CABI 2015). This species is native to South America, including Brazil, and was introduced into several countries as an ornamental plant (Heywood et al. 2007). In January 2016, several plants of *A. cordifolia* showing a foliar spot were observed in Jaguariúna, São Paulo (Southeast Brazil). The symptoms were initially characterized by small circular brown dots, with reddish margins, sometimes becoming confluent, later evolving to large blighted areas, sometimes with concentric rings and profuse fungal sporulation, resulting in early foliar abscission (Fig. 1).

Samples were collected and examined under a stereomicroscope. A dematiaceous hyphomycete was consistently found

associated with the observed symptoms. Using a sterile needle, the fungal structures present on the surface of the foliar spots were aseptically transferred to potato-dextrose-agar (PDA), which were then incubated at 25 °C for two days. The resulting colonies were aseptically transferred to PDA to obtain pure cultures. The two isolates obtained were deposited in the culture collection of the Embrapa Meio Ambiente (Coleção de Culturas de Microrganismos de Importância Agrícola e Ambiental) as accession numbers CMAA 1612 and CMAA1613.

Fungal structures obtained directly from symptomatic leaves, and also from cultures, were examined under a Leica DM2500 microscope. Measurements of relevant fungal structures were taken with the Interactive Measurement of the LAS® software v. 4.1 package. DNA was extracted from the two isolates using the Ultraclean® Microbial DNA Isolation Kit (Quiagen, Carlsbad). Mycelium obtained from colonies growing on potato-dextrose broth were filtered and ground in a mortar and pestle using liquid nitrogen. The internal transcribed spacer rDNA region (ITS) was amplified using primers ITS1 and ITS4 (White et al. 1990). The amplified PCR products were purified according to the ethanol precipitation method of Green and Sambrook (2012) and sequenced using the Big Dye® Terminator v 3.1 Cycle Sequencing Kit. Sequencing was performed by Instituto de Biociências of Universidade de São Paulo using the ABI 3730 DNA Analyser. ITS sequence of isolates CMAA 1612 and CMAA 1613 were deposited in GenBank as accession numbers MF196163 and MF196162, respectively. Pathogenicity tests were performed depositing 7-day-old mycelial discs of isolate CMAA 1613, grown on PDA, on leaves of *A. cordifolia* (Basellaceae), *Portulaca oleraceae* and *P. grandiflora* (Portulacaceae) and *Zigocactus truncatus* (Cactaceae), which were then maintained in a dew chamber, at 25 ± 2 °C and RH > 90%, for 72 h.

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Fig. 1 Symptoms of *Dichotomophthora* sp. on *Anredera cordifolia*. **a** Initial symptoms; **(b)** Advanced symptoms leading to early senescence; **(c)** Earlier senesced leaves under high humid conditions; **(d)** close up of foliar spot showing the pathogen signals (conidiophore and conidia)

The conidiophores observed were pale brown to brown, erect, simple or irregularly branched, sometimes verruculose near the apex, up to 900 μm long and 9–12 μm wide, “conidiogenous head” usually divided 3 to 4 times (Fig. 2a), frequently proliferating to produce additional heads (Fig. 2b); conidia were initially subhyaline, becoming brown to reddish-brown, solitary, smooth to finely verruculose (Fig. 2c–e), 1–6-septate, ellipsoidal to cylindrical, sometimes slightly curved, $24\text{--}94 \times 10\text{--}17 \mu\text{m}$, with rounded ends (Fig. 2c). Abundant immersed sclerotia were formed on PDA, and also on oatmeal agar, after 20 days at dark, while in malt-extract-agar only few, scattered sclerotia were observed. Based on the above characteristics, the fungus was identified as belonging to the genus *Dichotomophthora*. Using the ITS sequence on a megablast search in the NCBI GenBank nucleotide database, the closest hits obtained were *Bipolaris* sp. SZ-2011 (GenBank JF506092; Identities = 521/526 (99%), gaps = 2/526 (0%)) which was isolated from *Basella alba* in Florida (Zhang et al. 2011) and *Dichotomophthora portulacae* strain MXC 13071707 (GenBank KJ696544; Identities = 466/470 (99%), no gaps) isolated from *B. rubra* in China (Jing et al. 2008).

Based on morphological characterization, the fungus herein described resembled *D. lutea* more than *D. portulacae*. *Dichotomophthora lutea* differs from *D. portulacae* mainly based on conidiophore length and terminal cells arrangement, and conidia width (de Hoog and van Oorschot 1983). However, later works have reported a great variability on conidiophores and conidia size of what they have called “*D. portulacae*”, overlapping species circumscriptions (Eken 2003; Jing et al. 2008). A third species, *D. cactacearum*,

known only from Brazil, can be easily distinguished based on morphological traits (Da Ponte and Freire 1972).

The *Dichotomophthora* sp., herein, was able to infect its original host (*A. cordifolia*) and also *P. oleraceae* and *P. grandiflora*. No symptoms were observed on *Z. truncatus* (Fig. 3). The fungus was reisolated from the symptomatic inoculated leaves and had the same morphology as described above, fulfilling Koch’s postulates.

As mentioned, the present fungus is morphologically distinct from *D. portulacae*, although we demonstrated that it can infect *Portulaca oleracea*, the original host from where *D. portulacae* has been described. On the other hand, although our specimen is morphologically close to *D. lutea*, there is no sequence of the type strains of *D. portulacae* (CBS 174.35) and *D. lutea* (CBS 145.57) available to compare and confirm that they are indeed two separated species. Furthermore, later works report high variability on conidial and conidiophore sizes of *D. portulacae*, so we decided not to apply, at this moment, a specific epithet to the fungus causing leaf spot on *A. cordifolia*. However, considering that *D. portulacae* and *D. lutea* are indeed distinct species, based solely on morphological traits, we conclude that the fungus causing the symptoms observed on *A. cordifolia* is likely to be *D. lutea*.

Since their original descriptions, *D. portulacae* and *D. lutea* have been reported on several other hosts, including members of the Cactaceae (Farr and Rossman 2017). However, although the genus *Dichotomophthora* was previously reported on other members of Basellaceae, to our knowledge, this is the first report of this genus on *Anredera* worldwide. Additionally, due to the morphological and ITS

Fig. 2 *Dichotomophthora* sp. isolated from *Anredera cordifolia*. **a** Conidiophores apices showing conidiogenous heads; **(b)** Proliferation of a conidiogenous head; **(c)** Conidia; **(d)** Conidium superficial view; **(e)** Conidium median view. Bars = 50 μ m (a); 20 μ m (b, d-e); 10 μ m (c)

Fig. 3 Pathogenicity test results of *Dichotomophthora* sp. on its original host – *Anredera cordifolia* **(a)**; *Portulaca oleracea* **(b)**; *Portulaca grandiflora* **(c)**; and *Zigocactus truncatus* – not pathogenic **(d)**. See text for incubation time and conditions

similarities we are convinced that the “*Bipolaris* sp.” reported by Zhang et al. (2011) occurring on *B. alba* in Florida and the “*D. portulacae*” reported by Jing et al. (2008) occurring on *B. rubra* in China, are the same species. Moreover, it seems that the fungus occurring on members of Basellaceae belongs to a distinct species within the genus *Dichotomophthora*. However, due to the lack of DNA data of the *Dichotomophthora* species described so far, this hypothesis cannot be corroborated without further studies.

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