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First report of grey mould caused by *Botrytis cinerea* on *Hibiscus acetosella*

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Abstract
Botrytis cinerea is reported for the first time causing grey mould of cranberry hibiscus, *Hibiscus acetosella,* in Brazil and worldwide. The fungus was characterised morphologically and an ITS sequence helped confirm its identity. The fungus was isolated in pure culture and its pathogenicity to *H. acetosella* was demonstrated.

Keywords Malvaceae · Foliage blight · Fruit rot · Vegetable

Hibiscus acetosella (Malvaceae), known as cranberry hibiscus (vinagreira roxa in Brazil), is a species native from tropical Africa, now broadly distributed and used as an ornamental, but also as a source of fibre, for the preparation of dishes and beverages and as a medicinal plant (Fagundes and Massunaga 2016). It is closely related to *Hibiscus sabdariffa*, having similar uses. However, little has been published about its cultivation and also about the pathogens attacking it and there is not a single record of a fungus associated to *H. acetosella* in Farr and Rossman (2018). In November 2017, diseased *H. acetosella* plants were observed in a demonstration area in the campus of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil). Flowers and stems were necrotic and covered with grey fungal colonies. Infected flower parts detached and adhered to nearby healthy leaves, where new fungal colonies developed, later leading to the development of leaf blight (Fig. 1a-b).

Samples of diseased inflorescences and leaves were collected, dried in a plant press and deposited in Herbarium of the Universidade Federal de Viçosa under the accession number VIC 44366. Fungal structures were scraped from the surface of the colonised tissue using a scalpel, immersed in a drop of lactoglycerol on a microscope slide over which a coverslip was placed, then examined under a light microscope (Olympus BX 53) equipped with a digital camera (Olympus Q-COLOR3). Additionally, conidia were taken from sporulating colonies on infected tissues with the help of a sterile fine-pointed needle and transferred to plates containing potato dextrose-agar (PDA). One representative pure culture was deposited in the culture collection of the Universidade Federal de Viçosa (Acc No COAD 2343). Colonies were described on PDA after incubation at 25 °C under a 12 h daily light regime for four days.

The fungus had the following morphology - conidiophores isolated, sub-cylindrical, branched apically, ending in inflated fertile heads, up to 2000 μm long, 12–17 μm diam, 4–23 septate, dark brown becoming paler toward the apices, smooth; conidiogenous cells apical, ampulliform, 12–30 × 5–13 μm, hyaline; conidia ellipsoid to obovoid, formed in groups on the surface of conidiogenous cells, 5–15 × 5–10 μm often with a protruding hilum, aseptate, subhyaline, smooth. (Fig. 1c). Colonies growing on PDA were fast-growing (77–79 mm diam after 4 days), flat or effuse, margins fimbriate, aerial mycelium felty, white, straw reverse, sporulation abundant. The morphology of the fungus was very similar to that of *Botrytis cinerea* (Ellis and Walker 1974).

Genomic DNA was extracted from a 7-day-old COAD 2343 colony grown on PDA. Extraction was performed with a Wizard® Genomic DNA purification kit (Promega, USA) according to the manufacturer's instructions. The ITS rDNA region was amplified using ITS4 and ITS5 (White et al. 1990) and the PCR conditions were: initial denaturation for 1 min at 95 °C, 38 cycles of 94 °C for 1 min, 60 °C for 50 s and 72 °C for 1 min, and an additional extension step of 72 °C for to 2 min. Amplicon were sequenced by Macrogen Korea (http://dna.macrogen.com/eng/) and deposited in GenBank (Accession No. MH255797). The

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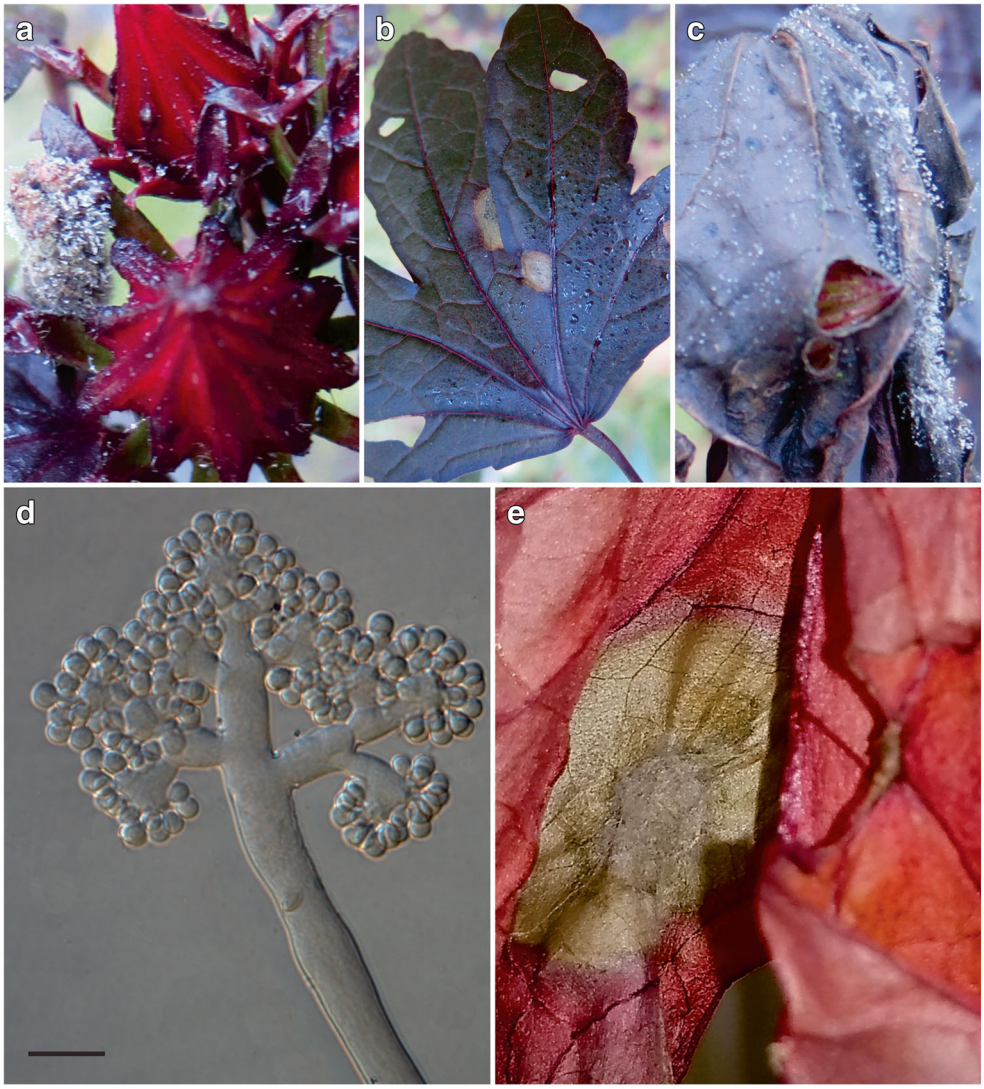

Fig. 1 *Botrytis cinerea on Hibiscus acetosella,* (**a-b-c**) Grey mould on fruits and leaves, (**d**) branched conidiophore apex with ampulliform conidiogenous cells bearing numerous conidia, (**e**) grey mould lesion with sporulation on a leaf. Bars = 10 μm

ITS sequence obtained was 99% identical to the sequence from *Botrytis cinerea* (KX443701).

Pathogenicity of isolate COAD 2343 was demonstrated: The fungus was grown on PDA plates for 7 days at 25 °C under a 12-h photoperiod. Two young healthy 80 cm high *H. acetosella* individuals were inoculated. Agar disks (4-mm-diam) colonised by COAD 2343, taken from the margin of actively growing colonies on PDA, were placed over the surfaces of selected leaves. Leaves exposed to pure PDA plugs served as controls. The plants were left in a moist chamber for 24 h at 25 °C and 12 h photoperiod and then taken to a greenhouse bench. The plants were observed daily for the emergence of symptoms. Three days after inoculation disease symptoms appeared (Fig. 1d), but only on leaves exposed to *B. cinerea*. Four days after the appearance of symptoms on inoculated leaves, colonies typical of grey mould developed on the necrotic lesions. Conidia harvested from the sporulating colonies were used to generate single spore in cultures on PDA which were identical to the cultures obtained from the naturally-infected vinagreira roxa plants in the field.

Botrytis cinerea is among the fungal pathogens having the widest known host-ranges (Ngugi and Scherm 2006). In Brazil, more than a hundred hosts of this fungus have been reported (EMBRAPA Recursos Genéticos 2018). This is the first record of *B. cinerea* causing grey mould on *H. acetosella* in Brazil and worldwide.

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