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First report of *Botrytis cinerea* causing gray mould on *Neomarica longifolia*

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Abstract
Gray mould of *Neomarica longifolia* (yellow iris) is reported here for the first time in Brazil and worldwide. The identity of the fungus was investigated and both morphology and molecular information demonstrated it to belong to *Botrytis cinerea*. Koch's postulates were performed demonstrating that *B. cinerea* was the etiological agent of the disease.

Keywords Etiology · Ornamental · Taxonomy · Yellow iris

Neomarica longifolia (*Iridaceae*), yellow iris, is a plant native from Brazil. It occurs in the sand dunes along the coast of the southeastern states and also in the state of Minas Gerais (Capellari 2000). According to Lorenzi (2013) it has been used as an ornamental because of its showy yellow flowers (Fig. 1a). Little is known about the pathogens associated with yellow iris. The sole mention of a disease attacking this species in the literature is that of *Sclerotium rolfsii* causing crown rot (Inácio et al. 2017).

In July 2017, plants of *N. longifolia* kept in a greenhouse in the campus of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil) for later use in an experiment, showed gray mould symptoms on flowers and flower buds (Fig. 1b).

Samples of diseased flower parts were taken to the laboratory for analysis. A dried sample was deposited in the herbarium of the Universidade Federal de Viçosa, as VIC 44317. A fungus was found in association with all diseased organs. A pure culture of this fungus was obtained by direct transfer of conidia from colonies on flowers onto PDA plates with the help of a sterile fine pointed needle. One representative culture was deposited in the local culture collection (Coleção Octávio de Almeida Drumond) - Acc. N° COAD 2291. Fungal structures were scraped from the infected flowers with a scalpel and mounted on lactofucsin and lactoglycerol and observed under a light microscope (Olympus BX 53) equipped with a Motic (Moticam 5) digital camera.

The fungus had the following morphology (Fig. 1c–e): Conidiophores subcylindrical, 300 2000 × 12.5–22.5 μm, branched, smooth, light brown, pale near the apex; Conidiogenous cells ampulliform, 10–20 × 7.5–12.5 μm; Conidia ellipsoid or subespheric, 6–12 × 6–10 μm, with slightly protruding hilum, smooth, light brown or subhyaline. This morphology was readily recognized as typical for *Botrytis cinerea* as described in Ellis and Waller (1974).

DNA extraction was performed with the Wizard Genomic DNA Purification Kit, by following the manufacturer's instuctions. Primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region of the 5.8S rRNA gene. Sequencing was performed by Macrogen Korea (www.macrogen.com). Sequences were edited and deposited in GenBank (Accession N° MH175197). A BLASTn search of sequences in GenBank indicated that COAD 2291 had 100% identity with the 199Jb14 isolate of *Botrytis cinerea* (Accession N° KU516438) as well as to many other *B. cinerea* isolates.

A conidial suspension of COAD 2291 was prepared by flooding a 7 days-old colony on PDA with 10 mL of sterile distilled water and scraping the surface of the colony with a rubber spatula. The concentration of the suspension was adjusted to 10^6 conidia/ml with the help of a haemocytometer prior to use. Two healthy *N.longifolia* individuals grown in 1 L plastic pots had their flower buds brush-inoculated with the conidial suspension. Two healthy plants treated with tap water alone served as controls. After inoculation, the plants were left in a dew

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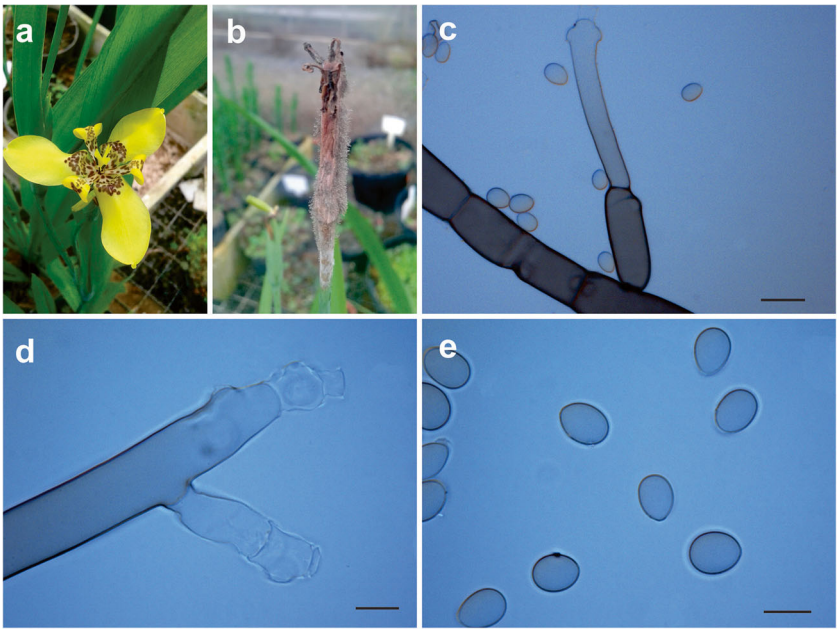

Fig. 1 **a** *Neomarica longifolia* bearing showy yellow flower, **b** Gray mould symptoms as observed on naturally infected *N. longifolia* flower bud with *Botrytis cinerea* colony, **c**, **d** Conidiophore and conidiogenous cells, **e** Conídia. Bars = 20 μm (**c**) and 10 μm (**d**, **e**)

chamber for 48 hs. After this period, the plants were transferred to a greenhouse bench and observed daily. Typical symptoms of the disease appeared two days after inoculation only on inoculated individuals.

Botrytis cinerea is a polyphagous pathogen attacking more than 230 different plant species (Horst 1998). Its wide range of hosts, its ability to survive as a saprophyte and the production of resistance structures make management of this pathogen difficult (Jarvis 1977). Little is known in terms of pathogenic fungi occurring on the entire genus *Neomarica* – even for the far more broadly cultivated *N. caerulea.* Only three records of fungi on *Neomarica* are listed in Farr and Rossman (2018) and only one includes an identification of the fungus at the species level. The specie is *Cercospora neomaricae* on *N. caerulea* (Macedo and Barreto 2008). The sole previous mention in the literature of a fungal pathogen of *N. longifolia* is also of a polyphagous pathogen – *S. rolfsii* (Inácio et al. 2017), but it is likely that further investigations will reveal more specialized pathogenic fungi on this host.

References

Capellari JRL (2000) Revisão taxonômica do gênero *Neomarica* Sprague (Tribo *Mariceae*, Subfamília *Iridoideae*, *Iridaceae*). DSc thesis, Universidade Estadual de Campinas

Ellis MB, Waller JM (1974) *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*). CMI Descriptions of pathogenic fungi and bacteria 431:1–2

Farr DF, Rossman AY (2018) Fungal databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/ Accessed 21 Jan 2018

Horst RK (1998) Compendio de enfermidades de rosas. GráficasUniversal, Brasil, Quito

Inácio AC, Rezende ALPS, Dresch LSK, Pimentel JP (2017) Techniques for inoculation of *Sclerotium rolfsii* on *Neomarica longifolia* and *Evolvulus pusillus* in Brazil. EC Microbiology 9:104–110

Jarvis WR (1977) *Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. Ontario: Research Branch-Canada Department of Agriculture, Canada

Lorenzi H (2013) Plantas para jardim no Brasil: Herbáceas, arbustivas e trepadeiras. Instituto Plantarum, Nova Odessa

Macedo DM, Barreto RW (2008) *Cercospora neomaricae* sp. nov. causing leaf spots on *Neomarica caerulea*. Australas Plant Pathol 37:581–583

White TJ, Bruns T, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18 315–322