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Botrytis cinerea causes grey mould on basil (*Ocimum basilicum*) in Brazil

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Abstract
Botrytis cinerea is reported for the first time causing grey mould on basil (*Ocimum basilicum*) in Brazil. Morphological and molecular features served to identify the fungus and its pathogenicity to basil was confirmed through inoculations under controlled conditions.

Keywords Condiment · Disease occurrence · Lamiaceae · Necrotrophic fungi · Vegetable

Basil (*Ocimum basilicum*)—alfavaca or manjericão in Brazil—is a condiment herb of the Lamiaceae which is native from tropical Asia. It is cultivated worldwide. In Brazil it is grown both in domestic and commercial vegetable gardens and sold fresh in the markets or to the industry for a broad range of uses, including as a medicinal herb (Lorenzi and Matos 2002). Nevertheless, relatively little has been published in the Brazilian literature about this crop's plant pathogens. Although Mendes and Urben (2020) listed 24 fungi found in association with basil in Brazil, the list included many saprophytes and fungi identified only at the generic level; and records are mostly unsupported by reference material. Also, the pathogenic status of most of these fungi has not been demonstrated experimentally. Recently, two diseases have been reported in detail on this host in Brazil by our research group: *Corynespora cassiicola* leaf spot (Pereira et al. 2019) and downy mildew caused by *Peronospora belbahrii* (Silva et al. 2019).

In July 2017, basil plants in a plot maintained in the Infectarium (plant disease demonstration garden) of the Departamento de Fitopatologia of the Universidade Federal de Viçosa (DFP, UFV) (Viçosa, state of Minas Gerais, Brazil) were found with flower, leaf and stem necrosis. Initial lesions on leaves were circular, 2–18 mm diameter, but developed quickly into irrregular blighted areas that coalesced and led to death of the leaves. The disease progressed further to produce a dieback of entire stems and the death of plants. This was accompanied with an abundant greyish sporulation on necrosed tissues, typical of grey mould symptoms (Fig. 1a-c). Fresh samples were collected and observed under a dissecting microscope (Olympus SZX7). A representative sub-sample was dried in a plant press and deposited in the local herbarium (Herbarium VIC) of the UFV – Acc. No VIC 47,344.

Conidia were transferred from sporulating colonies onto potato dextrose-agar (PDA) plates and homogenous pure colonies were obtained. Colonies on PDA (grown at 25 °C under a 12 hs/day light regime) were fast-growing (6.1 cm diam in 7 days), circular, raised, white, cottony, with abundant sporulation (Fig. 1d). One representative culture was deposited in the local culture collection—Coleção Octávio de Almeida Drummond (UFV) – Acc. No COAD 2922.

Fungal structures were scraped from the surface of colonized stems and leaves and mounted in a drop of lactoglycerol on a glass slide over which a coverslip was placed. Observations were made using a light microscope (Olympus BX 51) equipped with differential interference contrast and to which a digital image capture system (Olympus Q-Color 3 ™) was attached. Morphology: Conidiophores cylindrical, septate, brown, smooth, branching terminally; conidiogenous cells, terminal, ampulliform, 10–40 × 7.5–10 µm, subhyaline, bearing numerous denticles; conidia ellipsoid to ovoid, synchronously formed on denticles, 7.5–15 × 5–7.5 µm, aseptate, subhyaline, smooth (Fig. 1e-g). This morphology was recognized as typical for *Botrytis cinerea*, as described by Ellis and Walker (1974).

Using a rubber spatula, mycelium was scraped from a 7 days-old COAD 2922 colony for DNA extraction with

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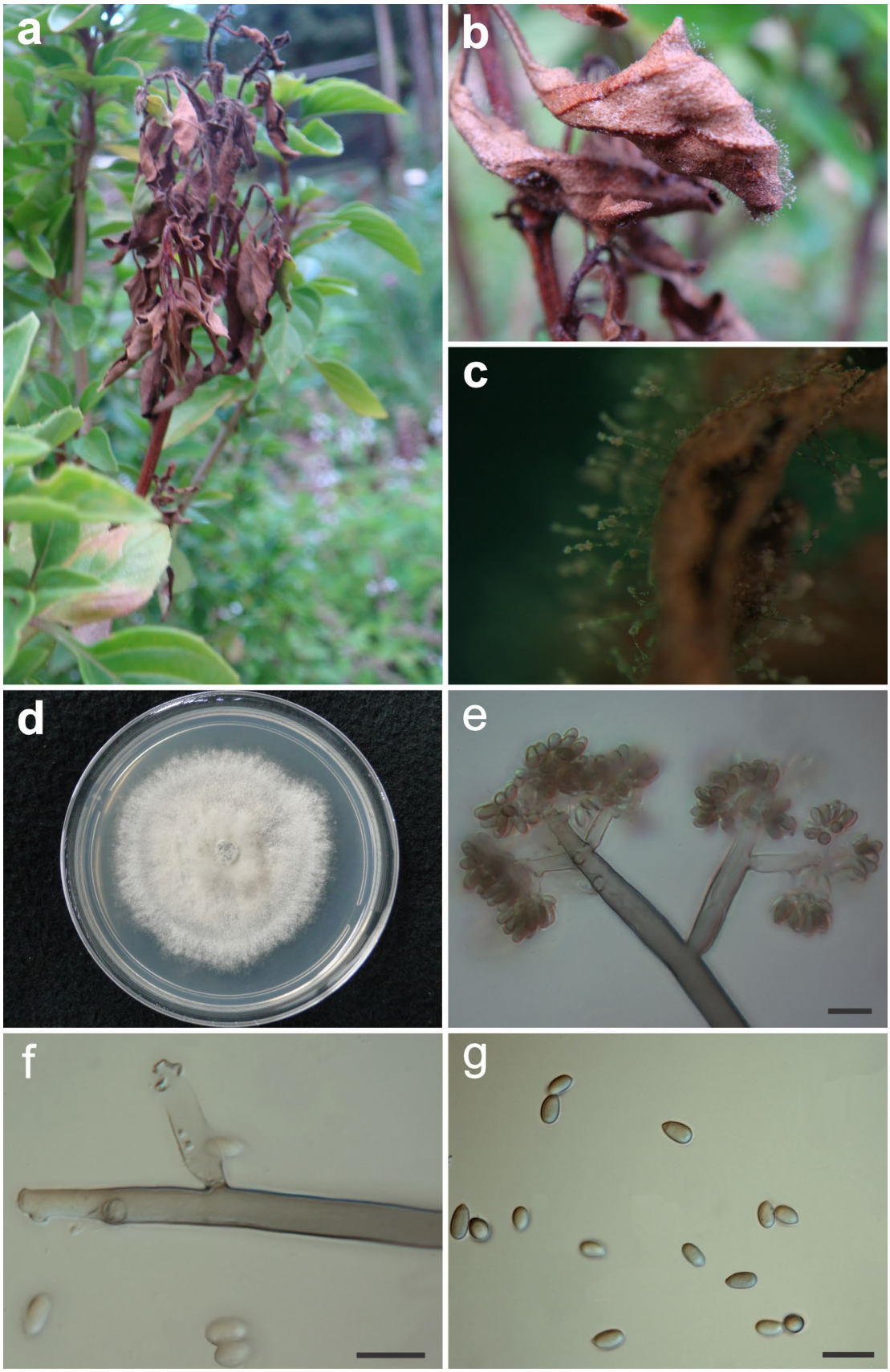
a
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◂**Fig. 1** *Botrytis cinerea* causing grey mould on *Ocimum basilicum*. **a**. Blight and branch dieback symptoms of plant in the field. **b**. Colony of *B. cinerea* on necrotic leaf. **c**. Close-up of colony of *B. cinerea* on dead basil leaf. **d**. Pure *B. cinerea* culture (isolate COAD 2922, from basil) on a 9 cm diam plate containing potato dextrose-agar. **e**. Conidiophore apex. **f**. Conidiogenous cells. **g**. Conidia. Bars = 50 µm **e**, 20 µm **f**, **g**

a Wizard® (Promega) genomic DNA purification kit, by following the manufacturer's protocol. The internal transcribed spacer (ITS) region was PCR amplified – with primers ITS4 and ITS5 (White et al. 1990); heat-shock protein 60 (HSP60) – with primers HSP60for and HSP60rev; and the second large subunit polymerase II (partial RPB2) - with primers RPB2for and RPB2rev (Staats et al. 2005). BLAST

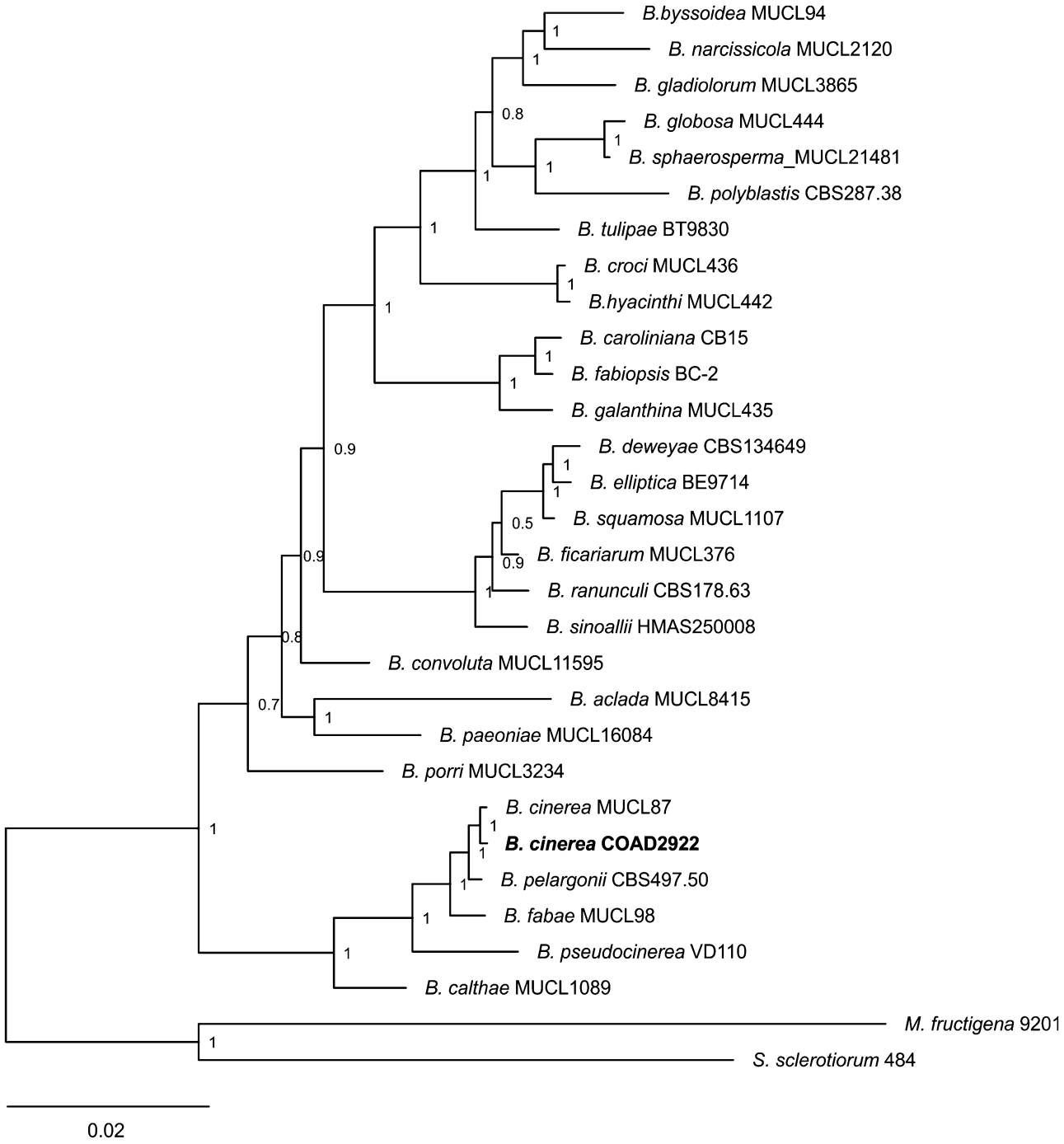

Fig. 2 Phylogenetic tree showing the relationship of *Botrytis cinerea* (isolate COAD 2922, from *Ocimum basilicum*) with other *Botrytis* spp., generated by Bayesian inference based on concatenated partial sequences of the G3PDH, HSP60 and RPB2 regions. *M. fructigena* (9201) and *S. sclerotiorum* (484) were used as outgroups. COAD 2922, from *Ocimum basilicum* is shown in bold

analysis of the COAD 2922 (GenBank Acc. No. MT089934, MW657229 and MW657228 respectively) revealed 100% homology with reference sequences of *B. cinerea*, previously deposited in GenBank (MN589849, MK791187 and MT604255 respectively) and this identification was further confirmed by phylogenetic analysis (Fig. 2).

In order to confirm the pathogenicity of COAD 2922 to basil, eight two months-old basil plants, grown in 0.5 L pots with a pasteurized mixture of soil, sand and manure (1:1:1) were sprayed with a conidial suspension (1.25×10^5conidia/mL) until runoff. Inoculated plants were left in a dew chamber for 48 hs and then transferred to the bench of a greenhouse. Four additional plants, prepared as described above and sprayed with distilled water, served as controls. Plants were observed daily for the emergence of symptoms. 72 hs after inoculation, typical grey mould symptoms appeared in all inoculated *O. basilicum* plants, but controls remained healthy. *Botrytis cinerea* sporulated on the necrotic tissues and conidia were transferred to PDA plates to produce colonies identical to those of COAD 2922), fulfilling Koch's postulates.

Botrytis cinerea is a polyphagous fungus that attacks hundreds of plant species worldwide. Although Farr and Rossman (2020) listed records of *Botrytis* sp. or *B. cinerea* on basil from Canada, Greece, Hungary, Italy, Poland and Turkey, this is the first report of *B. cinerea* causing grey mould on basil in Brazil. Within a short period of time three novel diseases of potential relevance to basil—an important condiment crop – have been found and reported for the first time in Brazil: *Corynespora* leaf spot, downy mildew and grey mould. These recent records were all based on observations made on plants growing in the Infectarium. This highlights both the gaps of our knowledge of pathogens of "minor crops" in Brazil and the practical relevance of the Infectarium as a sentinel for crop diseases, providing early warnings of new or unreported diseases which may threaten Brazilian agriculture in the future.

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