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Alternaria brassicae causes leaf spots on *Brassica juncea* in Brazil

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Abstract
Alternaria brassicae is reported, for the first time in Brazil, causing leaf spots on common brown mustard (*Brassica juncea*). A morphological and molecular identification was performed confirming the identity of the fungus and Koch's postulates were fulfilled through inoculation of healthy plants with a conidial suspension.

Keywords Common brown mustard · Condiment · Dematiaceous hyphomycete · Leafy vegetable · New report · Pleosporaceae · Target-spot

Common brown mustard (*Brassica juncea*: Brassicaceae) is a crop of major importance for the condiment industry, together with other *Brassica* and *Sinapis* spp. It is used as a leafy vegetable, medicinal plant and a source of oil among other things (Anjum et al. 2012; Caballero et al. 2015; Kinup and Lorenzi 2014; Wójciak and Dolatowski 2016). In Brazil, common brown mustard (CBM) cultivation remains mostly restricted to its use as a vegetable crop; however, seed is being imported for the preparation of mustard dressing and other preparations by the food industry. In order for Brazil to become a producer of mustard seed rather than an importer, we need to produce healthy mustard plants, which requires knowledge of various pests and pathogens affecting these plants and their management. Only powdery mildew fungi have been reported on CBM in Brazil (Mendes and Urben 2020).

In October 2019, leaf spots were detected on a plot of *B. juncea* plants in the disease garden (Infectarium) of the Departamento de Fitopatologia of the Universidade Federal de Viçosa. (Fig. 1a). Leaf spots started as minute greyish brown punctuations that grew becoming concentric spots, up to 0.5 mm diam., surrounded by yellow zones, which coalesced leading to the death of leaves (Figs. 1b, c). A sample of diseased leaves was collected for examination in the laboratory. Observation under a stereoscopic microscope (Olympus SZX7) revealed that the necrotic tissues of the leaf spots were regularly colonized by a dematiaceous fungus. A representative sample of the diseased plant was dried in a plant press and deposited at the local herbarium at Universidade Federal de Viçosa (accession number VIC 47379).

Fungal structures were scraped from lesions and mounted on lactoglycerol slides for observation under a light microscope (Olympus BX 51) equipped with an Olympus Qcolor3 ™ camera. The fungus had the following morphology: Condiophores isolated, subcylindrical, straight to flexuous, 71–168 × 6–10 μm, unbranched, 4–8 septate, light brown to brown; conidiogenous cells, subcylincrical, terminal, 7–37 × 5–8 μm; conidiogenous locus, 2–5 μm diam., thickened, darkened; conidia, produced singly or in short chains, obclavate, 78–174 × 10–21 μm, 7–17 transversely, 0–4 longitudinally septate, base rounded, 3–7 μm diam., apex beaked, hilum darkened and thickened, light brown to brown, smooth (Fig. 1d, e).

Pure and homogeneous cultures were obtained by transferring conidia to plates containing potato dextrose-agar (PDA) with a sterile thin-tipped needle. A representative isolate was deposited in the local culture collection – Coleção Octavio de Almeida Drummond (COAD) – under the accession number 2991. COAD 2991 was grown for seven days at 25 °C, under a 12-h light regime, on PDA, V8 agar, oatmeal-agar and also potato carrot-agar (PCA). Cultures were described according to the terminology of Crous et al. (2009) and Rayner (1970). Cultures were relatively fast-growing (up to 5.7 cm diam in 7 days), raised, flat or effuse, edge entire, cottony, white with mouse gray to pale mouse gray center, followed or not by an olivaceous buff ring and buff periphery; poor or no

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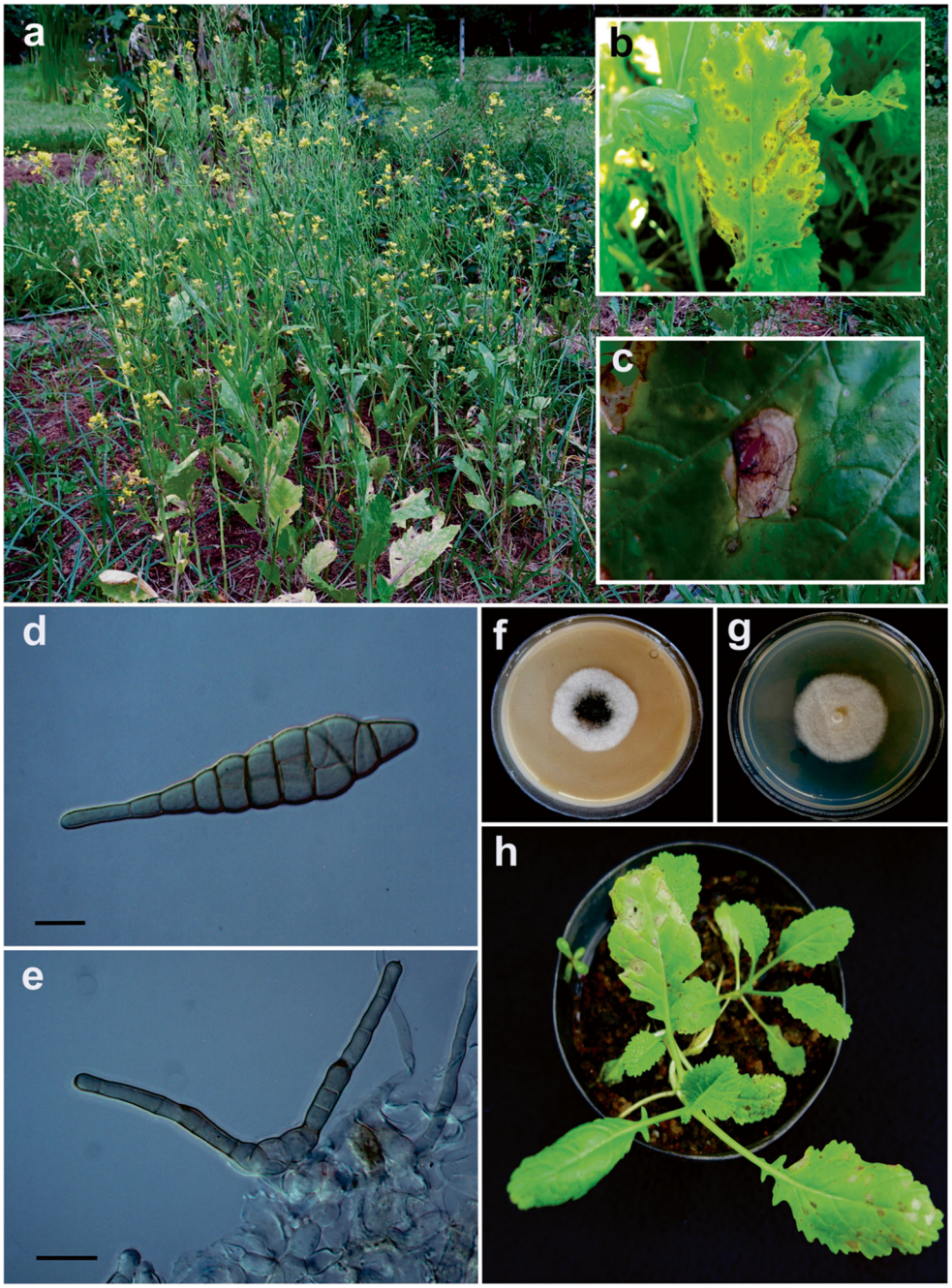

Fig. 1 *Alternaria brassicae* on *Brassica juncea* (common brown mustard - CBM), **a.** Plot of leaf spot-bearing CBM at the Infectarium (plant disease garden) of the Departamento de Fitopatologia - Universidade Federal de Viçosa (Brazil), **b.** Close-up of a CBM leaf bearing severe leaf spot symptoms at same location, **c.** Typical target-spot symptom on CBM leaf, **d.** Conidium, **e.** Conidiophores, **f.** *A. brassicae* COAD 2991 colony on V8 juice-agar, **g.** Colony on PDA, **h.** CBM 30 days-old test plants 8 days after inoculation with *A. brassicae*. Bars = 20 μm

sporulation (Figs. 1f, g). Morphological and cultural features of COAD 2991were recognized as equivalent to those described for *Alternaria brassicae* (Simmons 2007).

In order to further confirm the morphology/culture based identification, COAD 2991 was grown in potato-dextrose broth (PD) at 25 ± 2 °C under a 12-h light regime for 5 days. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. The *Altα1* genomic region of the isolated rDNA was PCR amplified with the Alt-for / Alt-rev primers Hong et al. (2005) and sequenced by Macrogen (Korea). The resulting sequence was deposited in GenBank (Acc No MT268533). The sequence obtained from COAD 2991 was found to have 100% identity with A*lternaria brassicae* sequences previously deposited in GenBank, namely LC481640 (Nishikawa and Nakashima, 2020) and AY563309 (Hong et al., 2005).

In order to verify the pathogenicity of COAD 2991 to CBM, five healthy 30-day old *B. juncea* were grown in pots containing a mixture of soil and substrate (3:1). Five CBM plants were used as controls. Test plants were inoculated with

a 1×10^5 conidia/mL suspension of COAD 2991. The conidial suspension was prepared as follows: COAD 2991 was grown in plates containing V8 juice-agar at 25 °C under a 12-h light regime for 12 days. After this period, plates were flooded with a 0.1% Tween 20 solution and the sporulating surface of the medium was scraped with a rubber spatula. The resulting conidial suspension was adjusted to the required concentration with the help of an hemocytometer. The suspension was then sprayed onto the abaxial surface of the leaves until runoff with a hand spray. Control plants were sprayed with sterile water only. All plants were placed in dew chamber for 48 h and later transferred to a greenhouse bench at ca. 25 °C and irrigated twice a day. Symptoms appeared 48 h after inoculation (incubation period) in all inoculated plants (Fig. 1h). Eight days later (latent period), fungal structures were observed on larger lesions. Controls plants remained healthy. *Alternaria brassicae* was re-isolated from necrotic tissues on the inoculated plants and pure cultures having identical morphology to COAD 2991 were obtained, fulfilling Koch's postulates.

Alternaria brassicae has been previously reported on *B. juncea* from Canada, China, India, Korea, Russia, Thailand, USA and the West Indies, according to the records in U.S. National Fungal Collection (Farr and Rossman 2020). This is the first record of *A. brassicae* causing leaf spot on *B. juncea* in Brazil.

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