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First report of *Passalora rosicola*, the cause of leaf spots on *Rosa multiflora* in Brazil

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Abstract *Rosa multiflora* is a species broadly used in Brazil as rootstock for the rose industry, nevertheless, little has been investigated on its pathogens. *Passalora rosicola* was collected in association with a common leaf spot of this host. Here, the fungus is fully described and its pathogenicity to *R. multiflora* is demonstrated for the first time.

Keywords Cercosporoid · Hyphomycete · Ornamental plant · Rootstock · Rosaceae

Rosa multiflora (Rosaceae) is a rose native from eastern Asia grown ornamentally and broadly used as rootstock for grafting commercial rose varieties for the cut flower market (Melida 1980). Rose hybrids are the main varieties in the cut flower market in Brazil and worldwide (Landgraf and Paiva 2005).

In September 2016, rose plants grown in a demonstration area in the campus of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil) were observed bearing severe leaf spot symptoms. Lesions started as small dark brown purplish dots that became circular with a greyish centre surrounded by a purplish brown rim, 1–4 mm diam, leading to yellowing of leaves and premature defoliation (Fig. 1a–b).

Samples were collected, dried in a plant press and deposited in the local herbarium (Herbário da Universidade Federal de Viçosa) under the Acc. number VIC 44117. A dematiaceous fungus was regularly found sporulating at the center of the lesions and isolated by aseptic transfer of conidia onto PDA plates with a sterile fine pointed needle. A representative isolate was deposited in the culture collection of the Universidade Federal de Viçosa as COAD 2177. Colonies were described on PDA after incubation at 25 °C under a 12 h daily light regime for 14 days. Fungal structures were scraped from the surface of the infected leaves, mounted on lactoglycerol and observed under a microscope (Olympus BX 51).

The fungus had the following morphology: Mycelium intra and intercelular, branched, septate, 2–6 μm diam, pale brown. Stromata subepidermal, irregular, 7–45 × 20–43 μm, composed of dark brown *textura angularis*. Conidiophores amphigenous, fasciculate, cylindrical, 45–148 × 2–5 μm, 0–2 septate, greyish brown at base becoming paler towards the apex, smooth. Conidiogenous cells terminal or intercalary, cylindrical, geniculate, 3–15 × 2–5 μm, pale brown; conidial scars thickened and darkened. Conidia isolate, subcylindrical to obclavate, straight, 22–65 × 2–8 μm, apex round, base obconic, 0–3 septate, pale brown, smooth; hilum thickened and darkened 1–3 μm diam. (Fig. 1c–e). In culture: Colonies very slow-growing (15–19 mm diam after 14 days), convex with papillate surface, irregular margins compressing the medium, aerial mycelium velvety, iron grey, dark mouse grey reverse; not sporulating. Morphology of the fungus placed it in the fungal genus *Passalora.*

Genomic DNA was extracted from a 7 day-old colony grown on PDA of isolate COAD 2177. Extraction was performed with a Wizard® Genomic DNA purification kit (Promega, USA) according to the manufacturer's instructions. The following primers were utilised for PCR amplifications: LR0R and LR5 (Vilgalys and Hester 1990) for the Large Subunit (LSU) region of ribosomal DNA (rDNA), and ITS4 and ITS5 (White et al. 1990) for the Internal Transcribed

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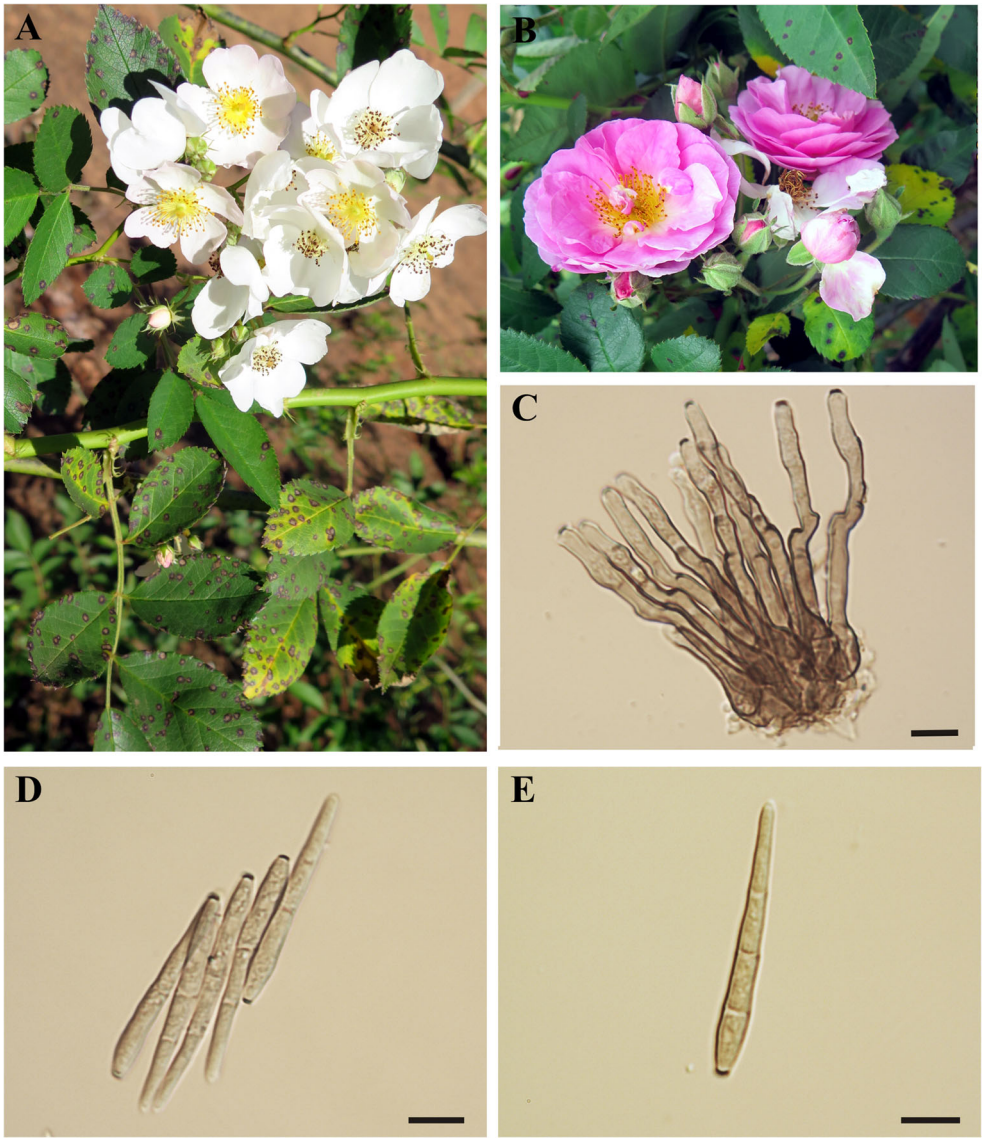

Fig. 1 *Passalora rosicola* on *Rosa multiflora,* (**a-b**) infected plant bearing leaf spots, (**c**) conidiophore fascicle, (**d-e**) conidia. Bars = 10 μm

Spacer (ITS) region of rDNA. The protocols described by Zhang et al. (2009) were followed for the former DNA region and that described by Groenewald et al. (2005) for the ITS region. PCR product was sequenced by Macrogen Korea (www. macrogen.com). After processing the ITS and LSU sequences were deposited in GenBank under the accession numbers MF370214 and MF370215, respectively.

There are three species of *Passalora* described in association with *Rosa multiflora*, namely *Passalora rosigena, Passalora rosae* and *Passalora rosicola.* All of these taxa were described from China (Farr and Rossman 2016). Fungal records were searched for species of *Cercospora* and related genera on *Rosa* sp., and there were numerous records worldwide, including Brazil. Morphology of the fungus found in Brazil on *R. multiflora* was in agreement with those described for *P. rosicola* (= *Cercospora rosicola*) by Chupp (1954) and Hino and Tokeshi (1978). Based on morphology, the isolate from Brazil was not *P. rosigena*, which produces external mycelium bearing secondary conidiophores (Guo et al. 2003); a character absent from the specimens in the present study. The morphology also was not similar to *P. rosae* (Braun 1995), which differs from *P. rosicola* because it produces shorter and narrower conidia (15–50 × 2–4 μm). To date there is no DNA information available for any of the species of *Passalora* described on *Rosa* spp. in public sequence databases. A BLAST search of the NCBI GenBank database of LSU and ITS yielded two species of *Passalora* as closest matches, namely: *Passalora perplexa* (GU214459) [identity = 875/886 (99%), gaps = 0/886 (0%)], for LSU, and *Passalora sequoiae* (JX436780) [identity = 504/548 (92%), gaps = 14/548 (2%)]. Based on such results it is concluded here that the fungus collected on *R. multiflora*

in Brazil is *P. rosicola*. No fungal pathogens have been previously recorded on *R. multiflora* in Brazil and, although the literature accounts for the presence of *P. rosicola* (as *C. rosicola*) on *Rosa* sp. in Brazil (Hino and Tokeshi 1978) that report was not supported by herbarium material, culture description, DNA information nor accompanied by experimental demonstration of pathogenicity.

Pathogenicity of isolate COAD 2177 was demonstrated by inoculating 15 leaves of young, potted *R. multiflora* plants with culture disks taken from the border of actively growing colonies on PDA. These were placed individually on the adaxial side of healthy leaves, which were then taken to a dew chamber and left there for 48 h. Healthy leaves of a separate *R. multiflora* plant treated with sterile PDA disks on leaves served as control. All plants were taken to a greenhouse bench and observed regularly for the emergence of symptoms. One month after inoculation, lesions similar to those observed in the field appeared only on inoculated leaves. Such lesions were colonised by *P. rosicola* and pure cultures of the fungus with the same morphology as described above were obtained. To our knowledge this was the first time that pathogenicity of *Passalora rosicola* to *R. multiflora* is demonstrated worldwide.

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